Excitotoxicity and Human Disease: 
The CNS

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Concept of Excitotoxicity

- In the 1969, Dr. John Olney discovered that MSG could cause certain neurons to become excessively excited, to the point that they would quickly die within 1 to 2 hours of exposure.
- Since this early discovery, several compounds have been discovered to have this property- mainly acidic proteins such as glutamate and aspartate.
- Cysteine in the presence of bicarbonate is also excitotoxic. Homocysteine is also an excitotoxin.
Concept of Excitotoxicity

- Olney discovered that certain areas of the brain were more sensitive than others: **arcuate nucleus**, supraoptic nucleus, paraventricular nucleus **of the hypothalamus**, hippocampus, amygdala, locus ceruleus, etc.

- The animals developed a particular set of findings:
  - short statue, **gross obesity**
  - shrunken endocrine glands and significant reductions in neurohormones, especially **prolactin, FSH, LH, ACTH and HGH**.
Special Characteristics of Excitotoxicity

- The immature brain is 4X more sensitive to excitotoxins than is the mature brain.
- Increased sensitivity again arises in the aged brain.
- Humans are the most sensitive to oral MSG of any known species.
  - 5X more sensitive than mice
  - 20X sensitive than Rhesus monkeys
- The dose of MSG causing these lesion is equivalent to amounts humans receive in their diets.
Excitotoxic Disorders

• Acute disorders:
  – Seizures
  – Brain trauma
  – Strokes (ischemia)
  – Hypoglycemia

• Chronic Disorders:
  – Alzheimer’s disease
  – Parkinson’s disease
  – Huntington’s disease
  – ALS
  – Viral encephalitis
  – HIV dementia
  – Autism
  – Heavy metal poisoning
  – Aluminum toxicity
  – Fluoride toxicity
  – Mitochondrial disorders
  – Depression/anxiety
  – Schizophrenia
  – Bacterial meningitis
  – Prion diseases

• Metabolic Disorders
  – Hyperammonemia
  – Hyperglycemia
  – hypoglycemia
Importance of Excitotoxicity

- Effects on Brain Development
- Normal Functioning of the brain
  - Learning and memory
  - Attention
  - Limbic control
  - Regulation of other neurotransmitters
- Immune-excitotoxicity interactions
  (immunoexcitotoxicity)
- Pathophysiology of: Alzheimer’s and Parkinson’s disease
- Ways to reduce excitotoxicity
Factors in MSG Toxicity

• Humans absorb MSG much easier than animals
• Human blood levels after dosing have variable absorption levels-from 19-fold to 50-fold increases in blood levels.
• Stegink found that adding aspartame to MSG dosing the blood glutamate level.
• Repetitive dosing can cause perturbations in neuroendocrine system without microscopic damage to the hypothalamus.
MSG Toxicity

- Viral infections can elevate blood glutamate levels
- MSG can produce silent lesions in brain
- MSG alters brain serotonin, norepinephrine, dopamine, GABA and acetylcholine levels during brain development
- Glutamate levels in fetus are twice the level in mothers following maternal MSG feeding
  - Lowers seizure threshold
  - Impairs learning
  - Behavioral aggressiveness and anxiety
  - Even without damage under light microscopy
Glutamate and Brain Development

• Found that fluctuations in brain levels of glutamate were critical in brain development. High levels over short period needed to prune excess synaptic connections and remove redundant pathways.

• Lower levels stimulated neuron migration and pathway development and eventual consolidation of connections.

• Excessive glutamate (excitotoxins) during neuronal migrations can halt cell migration leading to grossly abnormal brain development- hererotopias and intracortical arrest.

• High excitotoxin levels can cause arrest of neuron migration at all levels, producing a wide spectrum of architectonic patterns seen in human malformation- microgyria, pachygyrias, double cortex and lissencephalies.
Circumventricular Organs
Neuroendocrine Effects of Excitotoxins

• Van den Pol, et al - demonstrated that glutamate was the dominant excitatory transmitter in neuroendocrine regulation. (Science 250:1990)

• Olney found that even in subtoxic doses (1/4 dose) induced rapid elevation of LH and prolactin and depressed growth hormone release.

• In fully toxic doses- depressed LH, TSH, ACTH, GH and prolactin

• Perinatal exposure to MSG caused shrinkage of ovaries and uteri and lower estradiol levels.
Excitotoxins and Gross Obesity

- In real life situation, pregnant women, infants and children are exposed to numerous sources of excitotoxins.
  - MSG and related disguised products
  - Aspartame-40% aspartic acid
  - Naturally occurring excitotoxins
  - Spontaneously generated excitotoxins- cysteine-S-sulfonic acid from sulfite interaction with cysteine. (10X more potent as an excitotoxin as glutamate.)
Excitotoxins and Gross Obesity: Summery of the Evidence

• Consumption of food-borne excitotoxins has increased dramatically over last 20 years.
• Additive toxic effects of subtoxic doses of individual excitotoxins becomes fully toxic.
• Transplacental concentration of glutamate assures maximum toxicity to unborn infant.
• Infant and small child 4X more sensitive than adult.
• Dramatic increased use of soy based infant formulas.
• Use of aspartame products by mother during pregnancy.
• Proven hypothalamic damage in areas known to produce gross obesity.
Individual Variations in Glutamate Blood Levels Following a Meal

- People with gout or migraine headaches have higher blood glutamate levels.
- Those with ALS have 2x higher glutamate blood levels after a meal containing MSG than normal.
- People with even subclinical viral infection have 10-fold higher glutamate than normal.
- People exposed to mercury have higher brain glutamate levels.
- Among adults, blood glutamate levels can vary from with same dose of glutamate.
- Some infants develop 50-fold elevations in blood glutamate with glutamate-containing foods.
- Humans eat larger meals, test animals nibble.
Glutamate Receptors

• Ionotropic
  – NMDA
  – AMPA
  – Kainate

• Metabotropic
  – Group I-III with eight subtypes

• Glutamate receptors are linked via lipid Rafts and protein Scaffolding and are inducible
Glutamate Receptors: Ionotropic

NMDA
- NR1
- NR2 A-D
- NR3 A,B

AMPA
- GluR1-4

Kainate
- GluR5-7,
  KA1, KA2
NMDA Receptor Physiology

- Ligand-gated ion channel
- Requires simultaneous activation by glutamate and glycine
- Magnesium blocks ion channel opening-relieved by depolarization
- Modulated by polyamines-spermine and spermidine
- Zinc and NO inhibit NMDA activity
Glutamate NMDA Receptor Subunits

• NR1 is seen in all NMDARs and accounts for most of receptor properties
  - exist in 4 forms (NR2A-D)
    - Interacts with NR1 for full activity
    - Heterogeneously distributed in brain
    - Accounts for variations in NMDAR function

• NR2C selectively made in cerebellum—less sensitive to glutamate antagonists

• Neonatal animals typically express NR2B and forms
Glutamate NMDA Receptor Subunits

• In adult:
  – NR2A predominates in forebrain, hippocampus, cerebellum,
  – NR2B highly represented in olfactory tubercle, hippocampus, olfactory bulb and cerebral cortex
  – NR2C- highest in cerebellum
  – NR2D-low levels in thalamus, brain stem, olfactory bulb and spinal cord.

• Functional receptors require NR1 and variable combinations of NR2 subunits
AMPAR Function

- Exist both presynaptically and post-synaptically
- Responsible for fast transmission
- Trafficking between intracellular pool and membrane play major role in development and synaptic plasticity
- Intracellular sites major pool of GluR1 and GluR2 subunits utilized by AMPAR
- Glutamate receptors regulate actin-based motility of dendritic spines and growth cones
- AMPAR stimulation completely inhibits motility of growth cones in hippocampus
AMPA Receptor Subunits

• Has four subunits (GluR1-4)

• GluR2 is mainly repressed during brain development

• GluR1/GluR2 associated with adult brain function

• GluR2/GluR3 clustered on post-synaptic membrane
AMPAX Receptor Function

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AMPAT Receptor

- AMPA receptors normally are not calcium permeable
- AMPA receptors that do not contain GluR2 subunit makes them permeable to calcium
- See decreasing GluR2 subunit expression in a number of neurological diseases (epilepsy, ALS, etc)
- AMPA receptors interact with NMDA receptors and can lead to excitotoxicity
Depolarization

Synaptic cleft

Kainate receptor

Glutamate

Ca^{2+}

Na^{+}

NMDA receptor

Mg^{2+}

release

Depolarization

AMPA receptor trafficking

PKA

Calcium influx

cAMP

Adenyl cyclase

PKC

Ca^{2+} Calmodulin

MAPK

CREB

CaMKII

Protein synthesis

Presynaptic neuron

Postsynaptic neuron
Glutamate Receptors: Metabotropic

- Metabotropic Receptors
  - Group I
    - mGlu 1,5
  - Group II
    - mGlu 2, 3
  - Group III
    - mGlu 4, 6, 7, 8
Metabotropic Receptors

- Group I (mGluR 1/5) generally enhance iGluR activity
- Group II (mGluR2/3) induce microglial activation, mitochondrial dysfunction and apoptosis. Induces release of TNF-\(\alpha\)
- Group III (mGluR 4,6,7,8) protect against microglial toxicity
- Group II mGluR are activated by sustained elevated levels of glutamate
How the Brain Protects Itself From Glutamate Excitotoxicity

Extracellular Glutamate

- Glutamate Transport Proteins: GLAST and GLT-1 (EAAT1-5)
- Glutamate/cystine $X_C$-antiporter
- Glutamine synthetase
- Glutamate dehydrogenase
- Glutamic acid decarboxylase

- Enters astrocyte
- Exchange of cystine for glutamate increasing astrocyte glutathione
- Metabolism of glutamate
Excitotoxicity

- Classic Excitotoxic Cascade
- $X_C$-Antiporter
  - Oxidative Stress
Xc- Antiporter
Glutamate Cystine
Glutamate Cystine
2-Cysteines
Glutathione
Glutamate Transporters

• EAAT1-5
• EAAT1 and EAAT2 are predominately (GLAST and GLT-1)
• EAAT3, EAAT4 and EAAT5 expressed in neurons throughout brain
• EAAT4 and EAAT5 are specifically located on Purkinje cells and retinal neurons
Glutamate Transporters

- Protein Kinase C and Protein Kinase A control transporter trafficking
- NMDAR interact with EAAT3 to control its surface expression
- With induction of LTP - glutamate uptake is markedly increased
- Transporter levels are altered by kindling, seizures and drug abuse
- Transporter activity necessary for synaptic independence and input specificity
Things Decreasing Glutamate Re-Uptake

- β-amyloid
- Oxidative stress
- Mitochondrial dysfunction
- Pro-inflammatory cytokines
- IL-1β
- TNF-alpha
- IFN-alpha
- Mercury
- Lead
- Aluminum
Immunoexcitotoxicity
The Aging Brain
Excitotoxicity and Neurodegeneration in the Aging Brain

• Most neurons receive synaptic input from glutamatergic neurons
• Aβ, dopamine, mutant huntingtin, mutant Cu/Zn-SOD all sensitize neurons to excitotoxins
• Susceptible neurons have high numbers of AMPA receptors and low levels of calcium proteins
• With aging see perturbations in Ca\(^{+2}\) homeostasis and mitochondrial/ER calcium handling
Excitotoxicity and Neurodegeneration in the Aging Brain

- Genes associated with PD, AD and ALS are related to Ca+2 perturbations.
- Mutations in \( \alpha \)-synuclein associated with perturbations in Ca+2 regulation.
- Dentate cells in cerebellum (protected in AD) have high levels of calbindin.
- Aged-related loss of calbindin linked to loss of basal forebrain cholinergic neurons and entorhinal cortex layer II in AD.
- Pyramidal neurons (vulnerable in AD) have little or no calbindin.
Aged Brain and Inflammation

- **Interleukin proteins**
  - IL-1β was elevated the same in young and aged
  - IL-6 much higher in aged

- **Plasma cytokines**
  - IL-6 higher in aged
  - IL-1β higher in young

- Plasma cytokine levels did not reflect brain cytokine levels

- LPS caused only transient sickness behavior
Aged Brain and Inflammation

• In older mice vs younger
  – 38 genes controlling inflammation either up or down regulated (control complement)

• After exposure to LPS (Lipopolysaccharide)
  – 903 genes upregulated in aged brain only
  – Transcripts for mRNA for IL-1β, IL-6 showed greatest increase in aged brains
  – Transcripts for APP processing, INF-γ increased in aged brain only
Aged Brain and Inflammation

• See acute cognitive impairment after systemic infection
• Inflammatory cytokines known to inhibit LTP
• IL-6 neutralizing antibody prolongs LTP and facilitates recovery from LPS-sickness behavior
• IL-1β and TNF-α can also induce cognitive defect
• Humans given very low doses of LPS have no sickness behavior but do have impaired declarative memory
CNS Immunity

- Microglia are the resident immune cell in the CNS
  - Resting (ramified) - basal cytokines and growth factors
  - Amoeboid (activated) - secretes cytokines, chemokines, complement, etc.
Activated Microglia

Microglia

- Reactive Oxygen & Nitrogen Species
- Lipid Peroxidation Products (4-HNE & Acrolein)
- Excitotoxins:
  - Quinolinic acid
  - Glutamate
  - Aspartate
Inflammation/Excitotoxicity Interaction

- COX-1 is constitutive in microglia
- COX-2 is inducible in glutamatergic neurons
- COX-2 induced by NFkB
- PARP-1 plays a central role in NFkB expression of COX
- iNOS, IL-1β, TNF-α and glutamate act via PARP-1 to induce inflammation
- Prostaglandins (PGE2) inhibit glutamate re-uptake
- With infarction see robust activation of COX-2
- Dramatic generation of ROS/RNS and LPO
- NMDAR activation stimulates COX-2 activity
Microglial Activation and Glutamate Receptors

- MSG can activate resting (ramified) microglia
- MSG shown to significantly increase brain alpha, IL-1β and IL-6 in neonatal rats
- See widespread loss of neocortical cells with accompanying increase in NR1 expression and expression at PND-14 (NMDA and AMPA receptors)
Microglial Activation

- Changes in cell phenotype and gene expression
- Expression of MHC I and II
- Expression of cell adhesion molecules
- Elevation of secretion of pro-inflammatory cytokines and chemokines
- Induction of iNOS and nNOS
- Release of ROS/RNS and LPO
- Release of excitotoxins
Microglial Neurotoxicity

- Release storms of $\text{SO}^\cdot$, $\text{H}_2\text{O}_2$, $\text{OH}^\cdot$, peroxynitrite and 4-HNE
- Release of excitotoxins: glutamate and quinolinic acid in excitotoxic concentrations
- Nitric oxide released from astrocytes and microglia
- Inhibition of mitochondrial respiration by NO
- Mitochondrial suppression stimulates glutamate release from synapse and dramatic elevation in glutamate excitotoxic sensitivity
Acute Microglial Activation
Inflammatory Mode

- Predominant Proinflammatory Cytokines
- Cytokines (TNF-α, IL-1β, IL-6)
- Chemokines (MAP-1, MCP-1)
- Excitotoxins (Glutamate, Aspartate, and Quinolinic Acid)
Acute Microglial Activation

Immunoexcitotoxicity (failure to switch into reparative mode)

- TNF-α/IL-1
  - Glutamate-Induced $X_c$ Antipporter Suppression and Fall in Glutathione
  - Aging Effects
- TNFR-1/NMDAR Crosstalk
  - TNF-α Glutaminase Enhancement
- Chonic Immunoexcitotoxic Enhancement

Quin/Glutamate

- Mitochondrial Dysfunction
  - ROS/RNS/LPP
  - Gasapse Activation
  - Neuronal Loss
  - Hyperphosphorylated Tau
  - Dysfunctional Neurotubule

Progressive Neurodegeneration

Tangled Tau Proteins

Neurotubule Subunits Fall Apart

Hyperphosphorylated Tau
Dysfunctional Neurotubule

Stabilizing Tau Molecules
Inflammation/Excitotoxicity Interaction

- Reduced energy generation by mitochondria increase the sensitivity of NMDA and AMPA receptors - induced excitotoxicity
- Quinolinic acid increases as much as with immune stimulation
- Subcytotoxic levels of glutamate can destroy synaptic connections and lead to dendritic retraction
Microglial Neurotoxicity

• NO competes with oxygen at cytochrome binding site suppressing mitochondrial respiration

• This increases NMDA receptor sensitivity dramatically

• Shown that even nanomolar concentrations of NO produce rapid and reversible inhibition of mitochondrial respiration in brain synaptosomes and astrocytes

• Also see a dramatic suppression of neuronal respiration after exposure to activated microglia
Microglial Neurotoxicity and Mitochondrial Respiration

- Inhibition of mitochondrial respiration using specific inhibitors
  - Early neuronal death secondary to excitotoxicity and blocked by MK-801
  - Prolonged mitochondrial suppression - necrotic death not related to excitotoxicity
  - Astrocytes are less dependent on mitochondrial respiration than neurons with glycolysis sufficient for astrocyte energy production
Microglial Neurotoxicity and Mitochondrial Respiration

- NO release causes immediate glutamate release from a culture of mixed neurons and astrocytes
- Glutamate levels undetectable before NO added
- Glutamate re-uptake prevented by ROS/RNS/LPO, mercury, aluminum and pro-inflammatory cytokines
TNF-α and Immunoexcitotoxicity

- Impaired glutamate uptake (EAATs)
- Up-regulation of glutaminase in astrocytes and microglia
- Increased glutamate generation from glutamine
- Elevation of extracellular glutamate

TNF-α

- Stimulates trafficking of AMPA receptors to synaptic membrane
- Increases internalization of GABA_A receptors
- Suppression of glutamine synthetase
- Glutamate elevation
- Increases expression of GluR2-lacking AMPAR (calcium sensitive)

Chronic neurodegeneration
Sources of TNF-α

- Both neurons and glia can produce TNF-α
- The main sources are microglia and astrocytes
- Blocking TNF-α also blocks AMPAR trafficking and obliterates effect on synaptic strength
- Cortical neurons less sensitive to TNF-α effect than hippocampal neurons
TNF-Alpha effects on AMPA Receptor Trafficking

• **AMPA receptor trafficking controls synaptic strength for LTP and LTD**

• ** Trafficking mediated by endocytosis and exocytosis at postsynaptic site**

• **Mediated by synaptic activity**

• **Dynamics of AMPAR trafficking is very complex**

• **Silent synapses have no membrane associated AMPAR. Movement to membrane “un-silences” these receptors.**
AMPA Receptor Trafficking
TNF-Alpha and Excitotoxicity

- **Glutaminase** converts glutamine to glutamate
- Using glutaminase-free medium abolished neurotoxic effect of TNF-α via microglia almost completely
- **Glutaminase inhibitor** suppressed TNF-α-induced glutamate release and neurotoxicity
- **LPS and TNF-α** both up-regulated mRNA expression of glutaminase in microglia
- TNF-α enhances excitotoxicity by stimulation of both the **TNFR1** and **NMDAR**
Microglial Activation and Cellular Energy

Low energy levels by inhibiting complex IV of mitochondria

• Conditioned media with LPS or TNF-α- treated microglia also induced rapid drop in intracellular ATP and induced mitochondrial dysfunction in neurons

• Low intracellular energy production dramatically increases sensitivity to excitotoxicity
Systemic Immune Activation

CNS Microglial Activation

Glutamate
Quinolinic acid
Arachidonic acid

Cytokines:
- IL-1β, TNF, IL-2, IL-6

Free Radical Generation: ROS and RNS

Peroxynitrite

Lipid peroxidation:
- 4-hydroxynonenal

Accelerated Excitotoxicity

Mitochondrial dysfunction

Synaptic dysfunction
Part II:
Alzheimer’s Disease and Immunoexcitotoxicity
Hippocampus
Pathophysiology of Alzheimer’s Disease

• Earliest change is synaptic disruption and neurite shrinkage
• Chronic microglial activation within affected areas
• Altered membrane phospholipids (DHA)
• Elevated homocysteine
• Endocrine dysfunction elevated LH
• Excitotoxicity
• Widespread oxidative stress and lipid peroxidation
• Elevated inflammatory prostaglandins

• Ref: Atwood CS et al. Neuroinflammation, 2nd Ed, 2003, pp249-266
Pathophysiology of Alzheimer’s Disease: Soluble vs insoluble Aβ

- Normally secrete low levels of soluble Abeta
- With aging begin to accumulate insoluble Aβ 1-42 (from neuron)
- Aβ1-4 is more abundant in AD brain
- Concentration of amyloid is not directly correlated with progression of AD
- Strong correlation between NFT and elevated soluble Aβ.

Things that stimulate APP processing

- Proinflammatory cytokines/prostaglandins
- MSG (dietary excitotoxins)
- Activation of NMDAR and mGLuR
- Mercury and aluminum
- Low DHA levels
- Estrogen loss
- Elevated levels of Lutenizing hormone
- Oxidative stress
Alzheimer’s Disease and Microglial Activation

- See high density of activated microglia in diffuse plaque and throughout brain
- Aβ peptide is neither necessary or sufficient for microglial activation
- Most dying neurons not associated with amyloid plaque
- Correlation between plaque burden and between Aβ1-42 and synaptic loss is rather weak

**Acute Microglial Activation**

**Inflammatory Mode**

- Released:
  - Predominant Proinflammatory Cytokines
  - Cytokines (TNF-α, IL-1β, IL-6)
  - Chemokines (MAP-1, MCP-1)
  - Excitotoxins (Glutamate, Aspartate, and Quinolinic Acid)

**Acute Microglial Activation**

- Immunoexcitotoxicity (failure to switch into reparative mode)
Alzheimer’s Disease and Microglial Activation

• See elevated IL-1β, TNF-α and IL-6 in plaques
• IL-1 appears early in plaque formation
• Microglia secrete IL-1 as an early event around diffuse plaque.
• IL-1 activates microglia and microglia secrete IL-6 the main stimulus to astrocyte activation
• Systemic infection can produce prolonged microglial activation

Alzheimer’s Disease and Microglial Activation

- **IL-6** is elevated in AD brain as early event
- Microglial activation is associated with accelerated APP processing
- Aβ peptide activates p38MAPK and this activates the microglia.
- Microglial NADPH oxidase generates superoxide and NO (peroxynitrite), which inhibits mitochondrial function and enhances excitotoxic sensitivity

Alzheimer’s Disease and IL-6

- Animals with overexpression of IL-6
  - Decreased dendritic arborizations
  - Loss of cholinergic hippocampal innervation
  - Astrogliosis and microglial activation
  - Deficit in LTP and memory

Alzheimer’s Brain

- Elevation in NFkB activation
- Dramatic increase in ROS/RNS and LPO in brain and systemically
- Increase in anti-inflammatory PPAR-γ
- Low levels of IL-10 (anti-inflammatory)
- Elevation in brain iron, aluminum and mercury
Alzheimer’s Brain

- Elevated protein oxidation and nitration levels
- Elevated AGEs
- Increased DNA oxidation
- Impaired glutamate transport
- Evidence of extensive excitotoxicity

Dysfunctional Glutamatergic system in AD

- GLAST and GLT-1 are abnormally low in AD
- NMDA receptor and mGlu5R activation stimulates APP processing
- See decreased NMDAR, but remaining receptors are overstimulated by glutamate
- Glutamine synthetase activity is significantly reduced in AD (oxidatively modified)
- Mixing Aβ solutions with GS produces aggregates of Aβ
- Non-toxic concentration of Aβ has synergistic effect on excitotoxicity
Homocysteine in AD

• Alone, homocysteine has little toxicity but in presence of excitotoxicity and oxidative injury, greatly accelerates both processes.

• Homocysteine is converted into homocysteine sulfonic acid and homocysteine sulfinic acid, which are more toxic than glutamate

Acute Microglial Activation

Immunexcitotoxicity (failure to switch into reparative mode)

- TNF-α/IL-1
  - Glutamate-Induced Xc⁻ Antipporter Suppression and Fall in Glutathione
  - Aging Effects
  - TNFR-1/NMDAR Crosstalk TNF-α Glutaminase Enhancement

- Quin/Glutamate
  - Mitochondrial Dysfunction
    - ROS/RNS/LPP
    - Caspase Activation
    - Neuronal Loss
  - Inhibition of Serine/Threonine Phosphatase

Chronic Immunexcitotoxic Enhancement

- Progressive Neurodegeneration

Stabilizing Tau Molecules

Hyperphosphorylated Tau Dysfunctional Neurotubule

Tangled Tau Proteins

Neurotubule Subunits Fall Apart
Chronic Microglial Activation

Mercury

Vaccines and infections

Excitotoxins

Androgens and LH

Inflammation:
- TNFR1
- PGE2

Excitotoxicity

Glutamate aspartate

ROS/RNS/LPO

Food allergens
- Gliadin
- Gluten
- Casein

Aluminum
Cadmium
Lead
Fluoride (?)
Chronic Microglial Activation

- Inflammatory cytokines
- Inflammatory prostaglandins
- Excitotoxins: Glutamate, Aspartate, Quinolinic acid

Mitochondrial Dysfunction

- Lipid peroxidation products: 4-hydroxynonenal
- ROS/RNS: peroxynitrite

Neurodegenerative diseases
Depression and Immunoexcitotoxicity
Depression and Immunoexcitotoxicity

• Depression and anxiety frequently co-exist
• Affects 20% of population and appears to be growing in incidence
• Risk appears to be determined early in life
• Twin studies show 30 to 40% genetic influence
• Those carrying the 5-HT transporter (5-HTT) gene variant have high anxiety as infants and children.
Depression and Immunoexcitotoxicity

- See shrinkage of hippocampus, also a deficit in working memory (even without atrophy)
- In primate, hippocampal circuits do not fully mature until adolescence
- Major depressive disorder (MDD) resembles sickness behavior
  - Aversion to food
  - Fatigue
  - Insomnia or daytime sleepiness
  - Irritability
  - Social disinterest
  - Memory difficulties
Depression and Immunoexcitotoxicity

• The behavioral effects of sickness behavior are secondary to inflammatory cytokines and excitotoxicity
• Ketamine shown to dramatically improve depression and long after medication was stopped
• Patients treated with inflammatory cytokines often develop MDD
Depression and Immunoexcitotoxicity

• See major depression with inflammatory diseases:
  – Rheumatoid arthritis
  – Cardiovascular disease
  – Type 2 diabetes

• With aging see increasing inflammation and increasing depression/anxiety

• Animals injected with IL-1β or TNF-α show depressive behavior
Depression and Immunoexcitotoxicity

- **Peripheral immune links to CNS:**
  - Vagal
  - Trigeminal
  - Humoral TLR on macrophages lining the CVO and choroid plexus
  - Cytokine transporter on BBB
  - IL-1 receptor on perivascular macrophages and endothelial cells

- **Subseptic doses of LPS induces expression of IL-1β, TNF-α in brain**
Depression and Immunoexcitotoxicity

- In older mice see an exaggerated sickness behavior on exposure to LPS
- Low IL-10 worsens response
- Mice carrying diabetic genes (db/db) respond to LPS with exaggerated sickness behavior
- Aging itself can prime microglia
- In aged see more prolonged and profound depression
- Lemstra et al first to show that systemic infection in humans can result in microglial activation.

Ref: Godbout JP et al. FASAB J
Depression and Immunoexcitotoxicity

- Inflammatory cytokines lower plasma tryptophan
- See increased tryptophan metabolism
- Increases quinolinic acid in brain (kynurenine pathway)
- Increases uptake of serotonin
- Chronic inflammatory cytokine elevation increase cortisol receptor resistance and this increases cortisol
- Cortisol enhances excitotoxicity in hippocampus
Depression and Immunoexcitotoxicity

- Antagonist of GluR 2,3,5 of mGluR has antidepressant effect
- Zinc acts as an antidepressant by suppressing NMDA receptor activity
- SSRI medications modulate AMPA receptors and this reduces depression
- Interaction with other neurotransmitters: dopamine, locus coeruleus (norepinephrine) and nAchR.
- Study of 38 depressed patients found higher glutamate and lower GABA in brain
Multiple Sclerosis and Immunoexcitotoxicity
Multiple Sclerosis and Immunoexcitotoxicity

- In animal model of MS, EAE, blocking AMPA/Kainate receptors significantly ameliorated the disease.
- It did so without reducing the intensity of the immune reaction.
- There is an extensive presence of AMPA receptors on oligodendroglia.
Multiple Sclerosis and Immunoexcitotoxicity

- See microglia activation at all active stages of multiple sclerosis
- IL-1ß in a mixed culture will kill oligodendroglia
- Antagonist of the AMPA receptor will prevent this
- TNF-α will also kill oligodendroglia (IL-1ß stimulates release of TNF)
- Source of IL-1ß and TNF-α is the microglia
Multiple Sclerosis and Immunoexcitotoxicity

- PET scanning using peripheral benzodiazapine receptor (PBR) on 7 healthy controls and 22 patients with MS
- During disease relapse see increased PBR scanning with disease progression
- See low levels of GLT-1 around active MS lesions
- This raises the glutamate level around the affected neuron fiber
Multiple Sclerosis and Immunoexcitotoxicity

- See absence of glutamine synthetase and glutamate dehydrogenase around active and chronically silent lesions
- This elevated local glutamate levels
- TNF-α elevates glutaminase and this increases local glutamate generation and secretion
- TNF-α also increases trafficking of AMPA receptors (increases excitotoxicity)
Protection from Immunoexcitotoxicity
Things That Enhance Excitotoxicity

- Low mitochondrial energy production
- Low magnesium in CNS
- Systemic immune activation
- Mercury (all sources- ionic most damaging)
- Histamine excess
- Fluoroaluminum, lead, cadmium, triethyl tin
- Pesticides/herbicides and neurotoxic chemicals
Protection from Excitotoxicity

• **Reduce Inflammation: Supplements**
  - Buffered vitamin C
  - Natural form vitamin E (high gamma-E)
  - Silymarin
  - Curcumin
  - Quercetin
  - Resveratrol
  - Ellagic acid
  - Boswellia
  - Magnesium citrate/malate
Protection from Excitotoxicity

- **Increase Cellular Energy**
  - Riboflavin-5-PO$_4$
  - Pyridoxal-5-PO$_4$
  - Niacinamide
  - Vitamin K
  - Thiamine (Benfotiamine)
  - CoQ10 (ubiquinol)
  - R-lipoic acid
  - Acetyl-L-carnitine
  - Acetyl-L-carnosine
  - Pyruvate
Protection from Excitotoxicity

• Reduce Immune Overactivity
  – Silymarin
  – Vitamin D3
  – Magnesium
  – Omega-3 oils

• Directly block excitotoxicity
  – DHA
  – Tetracycline antibiotics
  – Dextromethorphan
  – Prescription drugs