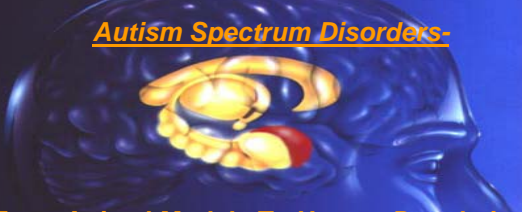


"Exploring Potential Gut-Brain Links in Autism Spectrum Disorders- From Animal Models To Human Populations"



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Website:
<http://psychology.uwo.ca/autism.htm>
 (peer reviewed publications, webcasts)

OVERVIEW

Clinical Presentation of Autism Spectrum Disorders- A systemic disorder?

Dietary Exacerbation of Autism- GI Factors Link to Antibiotic Associated Diarrhea

Evolutionary Ethobiology and the Human Microbiome
 Can infectious processes control host behavior? Are the Microbes in Charge?

Enteric Short Chain Fatty Acids- A Common Link?

Biological Effects- Brain, Gut, Immune, lipid metabolism, oxidative stress, glutathione, gene induction

Kilee Patchell-Evans Autism Research Group-multi-disciplinary

Using Animal Models to Study ASD's- rational study of environmental factors

- hyperactive/repetitive/perseverative/anti-social behavior
- brain electrical activity (seizure/movement disorder)
- neuropathology (neuroinflammation/neuroplasticity)
- metabolic/epigenetic
- link to human studies- genetic/acquired sensitive sub population?

DISCLAIMER

Research depicts studies using rodents with EEG electrodes/drug ports to measure brain activity and behaviour, with studies based on extensive biochemical and tissue culture research

Similar to human patients undergoing workup for surgical treatment of epilepsies

Research ethics in strict accordance with Canadian Council on Animal Care and University of Western Ontario Animal Use Committee

This basic science research in no way is intended to support any treatment claims by any groups not medically sanctioned by experienced physicians practicing evidenced based medicine in autism spectrum and related disorders.



Autism – A Brain Disorder of Repetitive Movement, Restricted Interests, Sensory Sensitivity and Impaired Socialization



Originally 1:10,000 (1950's)
Now 1 in 90 persons (males>females)

- Abnormal Social Interaction
- Speech and Language Difficulties
- Repetitive Stereotyped Movements
- Self-Injurious Impulsive Behavior
- Sensitivity to Sensory Input
- Savant Syndrome (rare)
- Regression in some patients

Comorbidities:
Seizure disorder
Gastrointestinal dysfunction
Immune/metabol. abnormalities CNS/GI*

Genetic < 5%
Genetic/environmental interactions?



**SIMPLE CAUSE OF COMPLEX DISORDER?
MULTIPLE CAUSES OF FINAL COMMON PATHWAY?**



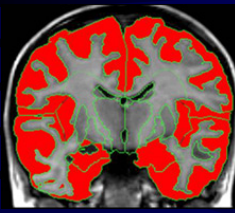
The Genetics of Autism

- Identical twin studies- 50-80% concordance - genetics and environment
- Multiple Chromosomes- 2,3,7, 15, 16, 17 X- chromosome mapping
- Multiple genes- brain development, neurotransmitters, language centres
- Intercellular connections- Neurexins
- Disorder of Gene expression (methylation, acetylation of histones)
- Met Receptor Tyrosine Kinase & Protein Kinase C beta 1 (brain, gut, immune)
- **85-97% NO DEFINED GENETIC CAUSE**
- Oversimplistic to say one specific genetic cause
- **Epigenetics- interaction with genes/environment**
- "Spontaneous (?)" genetic mutation
- Other genetic disorders where autism is associated
 - Fragile X**
 - Angelman/Prader Willi Syndrome**
 - Epilepsy- "tuberous sclerosis"*
 - Rett Syndrome
- Mitochondrial genetic disorder?

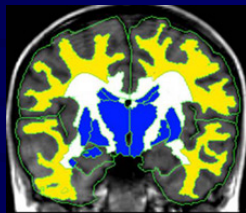
Role of Vaccine MMR- Controversial

- Epidemiological evidence not conclusive (sensitive subgroup?)
- Continued increase in ASD despite reduction in thimersol
- *Danger of tunnel vision - Many other factors occurring at that time period (paediatric infections, antibiotics, pathogen spread)*
- Lack of immunization = "home for viruses" and mutation
 - multiple children with developmental delay
 - i.e. congenital rubella syndrome
- Ongoing epidemiology- Queens prospective study (Dr. Holden)

Enlarged White Matter in ASD patients (Herbert)

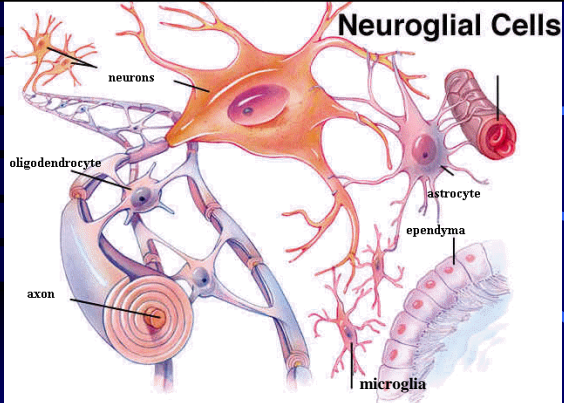


Grey matter atrophy



white matter hypertrophy (edema?)

Neuroglial Cells



Autism- A White Matter Disorder? Brain Interconnectedness

Neurodevelopment- "Lets Build a Brain"

- Complex development
- timing important
- Many neurons die

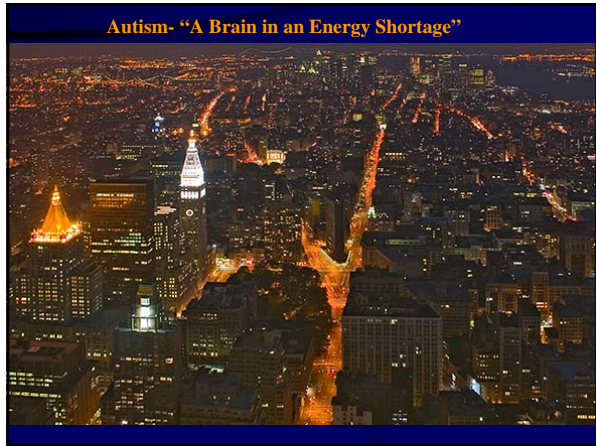
Genetic (instruction)
Environment

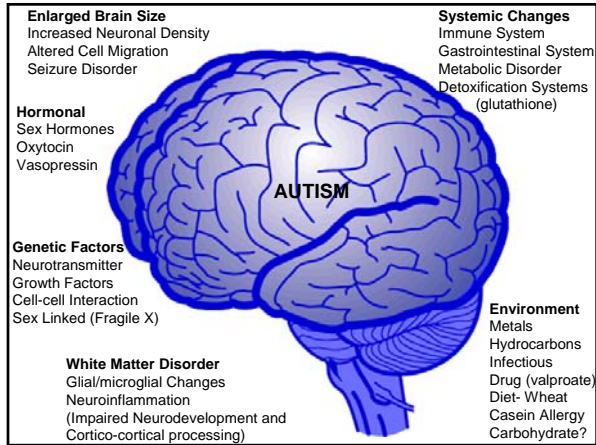
Insults:
Infection (virus)/inflammatory (IL-6)
toxins (alcohol)/metals/drugs(valproate)
(germ cell-fetus-neonate)

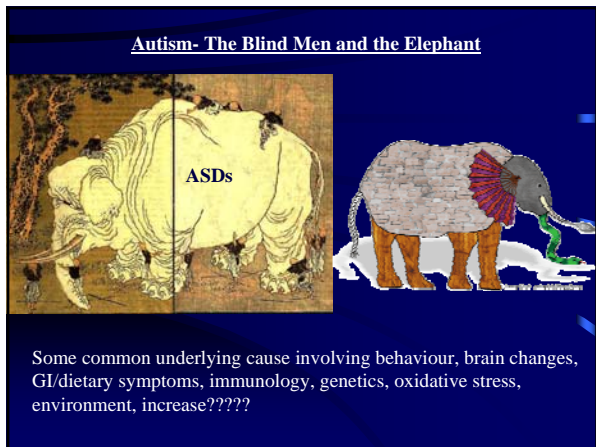
Cell to Cell Communication is
Important in the organization of the
developing nervous system

(programmed cell death and ordered cell migration)









**The Kilee Patchell-Evans
Autism Research Group**
THE UNIVERSITY OF WESTERN ONTARIO

*"Scientists Listening
to Parents"*

- The paradigm of understanding Autism is changing
- Autism is a whole body disorder with many potentially treatable features
- We are an international multi-disciplinary team of neuroscientists working towards a cure






The "Kilee Patchell-Evans Autism Research Group"
David Patchell-Evans- CEO GoodLife Fitness




Kilee Patchell-Evans Autism Research Group 2004




Using animal models to study the neurobiology of autism


Examining Animal Behaviour to Study Autism



Decreased/altered socialization
 fixation on objects
 sensitivity to sensory input
 repetitive behaviour/ seizure/dystonia
 aggression
 other factors normal/ improved?




Animal autism models
 Pre/post natal factors



Examine brain
 Development
 Electrical Activity
 Neuropathology
 Metabolic markers
 for subtle
 abnormalities

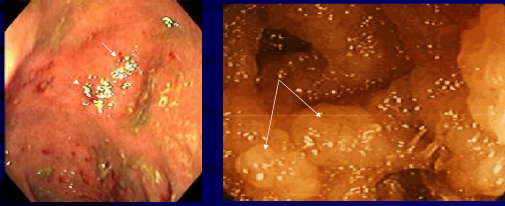
“GRAIFs” Gut Related Autism Inducing Factors
 Microbiome (10x host cells)



Bacterial metabolites- symbiosis/dysbiosis
 Opportunistic Infections- key risk factor
 i.e clostridia, yeast (chronic antibiotics)
 Cell wall- LPS, beta glucan- innate immunity
 Fermentation products of dietary carbohydrate
 - Short chain fatty acids*
 Barriers, variable metabolism
 Acquired/genetic (met receptor tyrosine kinase)
 Timing of exposure

Digestive Tract Pathology in Autism- Lymphoid Nodular Hyperplasia (Wakefield, Horvath)

Lymphoid Nodular Hyperplasia



Intestinal pathology on a subset of autistic patients
 Associated with regressive onset and GI symptoms
 Moderate inflammatory process (nonspecific?)
 Cause????

Can Enteric Bacteria Affect Brain Development/Behaviour?



Clinical- Food Craving/Symptom Worsening
 Gut changes (gluten/casein) poorly studied (antigenic mimicry)
 Early gut colonizers- alteration with antibiotics (increased incidence)
 "Leaky" or malabsorptive digestive tract (impairment of barriers)
 Production of bacterial metabolites (fuel for brain)
 Effect on Brain development, physiology, behaviour, immune function

Carbohydrate Craving, Diarrhea and Fecal Smearing in Autism



Behaviour facilitates growth and spread of autism implicated gut pathogens (clostridials)?

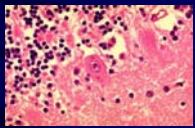
Pathogen affecting host behaviour

M. Herbert

Pathogen "Control" of Host Nervous System for Propagation



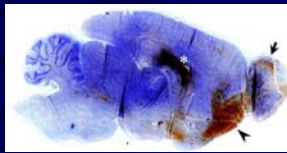
Cordyceps Fungus
Climbing (insects)



Rabies
biting (mammals)



Borna Disease



Brain specific
Mammals, birds
Nasal transmission
Movement disorder
Oral movements
"food in mouth"
Human infection?
(mood disorder
Schizophrenia)

Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Infection (PANDAS)



Associated with Group A Beta Strep infections
Clingy- OCD/Tick symptoms, relapsing remitting
Autoantibody to basal ganglia
Similar behaviour in family (sensitive population)

C. difficile

GM+, spore,
Toxin A (enterotoxin),
B (cytotoxin) binary?,
biofilm ("hiding")
Severe- pseudomembranous
Colitis
? Mild infections/carrier state
? age of infection
Finegold- regressive ASD

Clostridium Difficile – Epidemic

Genetics ARE important- Genetic mutations of infectious processes too!

Development of Agriculture and Animal Husbandry

Development of Cereal Diet, Animal Domestication & Urban Culture
Co-Evolution of Endogenous Florae? (J. Diamond/M. Pollan)
Cultural Taboos with cattle/dairy



“Urbanization of Cattle”- Crowding, Corn and Chronic Antibiotics
(clostridia, propionibacteria)



Evolutionary Psychiatry- population vs individual



Are the Microbes in Charge?

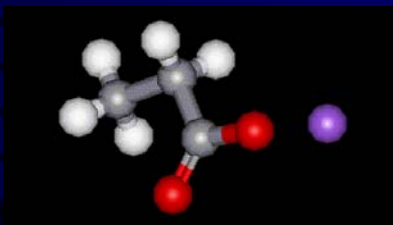
Some direct/ indirect advantage to behavioural trait

Autism in Somali Diaspora in North America



3% of general population, 35% of autism in some regions
All conceived in Receiving Country- NOT Somalia
Large exposure to antibiotic+++ gastrointestinal infections

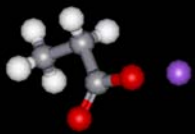
Propionic Acid- Neuroactive properties



Weak organic acid: lipid/water soluble
Uptake passive active (monocarboxylate receptors)
Intracellular concentration (intracellular acidification)
Unique CNS/GI immunological properties

Short Chain Fatty Acids – Propionic Acid

Propionate:



Byproduct of bacterial metabolism
Clostridium, propionibacteria (gut/acne)
Desulfovibrio, Bacteroidetes (Fingold)
(butyrate, acetate)- short chain fatty acids
Common preservative of wheat and dairy products

Increased by ethanol, B12/biotin deficiency

Variable metabolism of propionate in population – Multiple mechanisms and multiple clinical presentation shares similarities with autism- underreported???

Role of diet, gut bacteria/barriers and “sickness” in propionate levels (other short chain fatty acids and metabolites)

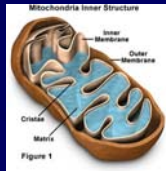
A Review of Propionic Acidemia:

- Part of a family of metabolic disorders (methylmalonic acidemia)
- propionyl CoA carboxylase, multiple carboxylase, biotinidase deficiency
- considerable polymorphisms (chromosome 3 and 13) – underreported
- Elevated in other organic acidemia, biotin/B12 deficiency, alcohol
- Developmental delay, seizure, movement disorder, GI disturbances.
- Acidosis/ propionate excretion may or may not be present

A Review of Propionic Acidemia (cont):

Mechanisms:

- Mitochondrial disorder leads to increased propionate/ propionyl CoA.
- Intracellular accumulation of short chain fatty acids leading to acidosis.
- Increased nitric oxide, peroxide, impaired -SH,
- **NB- Carnitine depletion – mitochondrial uncoupling**
- Glutamate/dopamine 5HT release
- lipoperoxidation (membrane damage)
- Gene expression (Tyrosine OHase, enkephalins)
- histone deacetylase inhibitor (gene expression)
- “Sensitivity to metabolic stress”



Rodent Model of Autism- Effects of Propionic Acid Administration
(MacFabe et al, Behavioural Brain Research 2007)



Autism Model Rodents – Propionic Acid- behaviour/EEG

PBS



- Injected into cerebral ventricles
- NB buffered to pH 7.5
- Reversible repetitive behaviour
- Fixation on objects
- Seizure +/-behaviour cortex
- Subcortical spiking

Propionic Acid (PPA) Autism Model



Effect immediate, transient (45min) but some permanent

Automated Behavioural Monitoring (Ethovision):

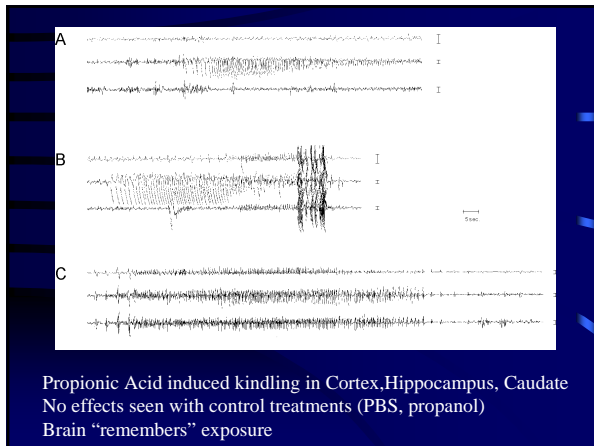
- Computerized long term quantification of movement
- Combined with drug administration, brain electrical activity
- Repetitive behaviour

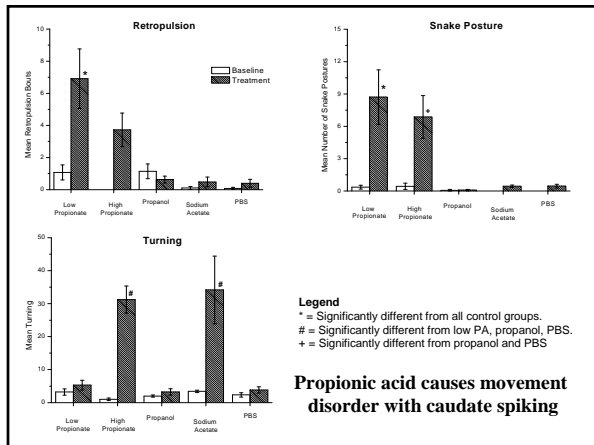
Vehicle

Propionic Acid Autism Model

Intraventricular Propionic Acid- “ritual”

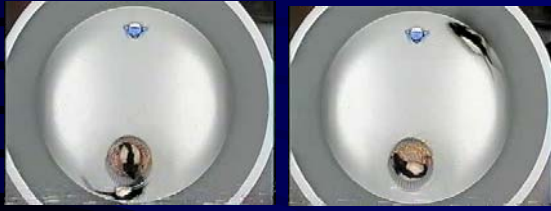
Hippocampal EEG- Repetitive motor loop
Normal EEG







Control/PPA rat "introduced" to normal rat (in cage)

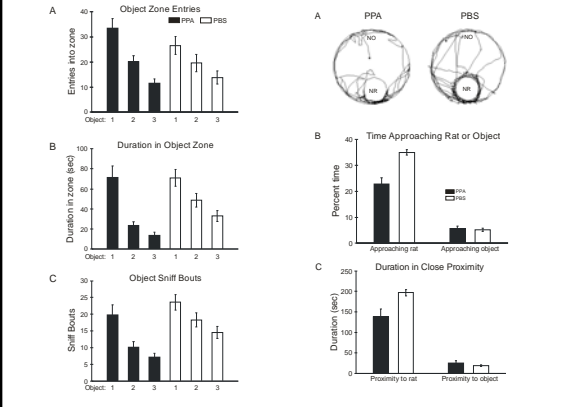


PBS (control rat)
Normal social interaction

PPA rat "ignores" normal rat

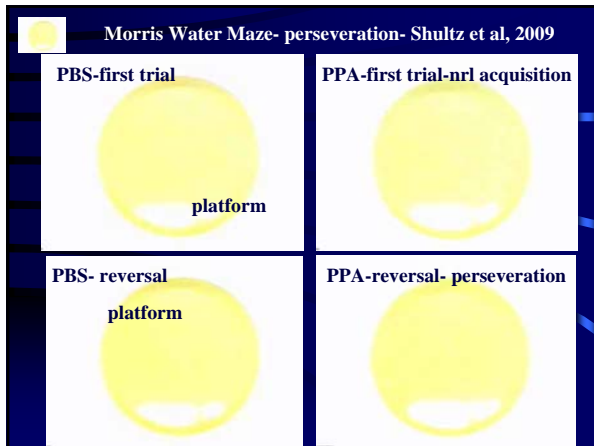
MacFabe et al; Behavioural Brain Research (2010)

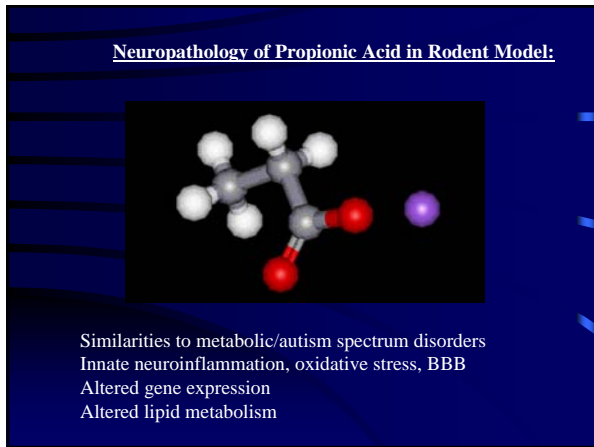
PPA Rats Prefer "Favourite Objects" to other Rodents (MacFabe et al, 2010 BBR)

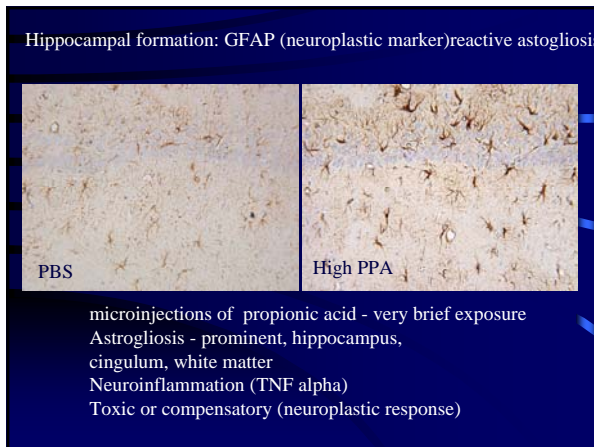




Long term effects- Ethovision- stereotypies/ object fixation

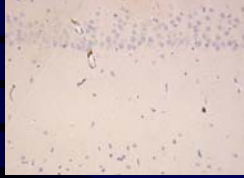




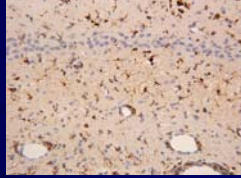


Results – CD68 Microglia – 14 day

Control (PBS)



Propionate (PPA)



PPA increases activated microglia (neuroinflammation)
Nitric oxide, cytokines
Endovascular involvement (microcirculation/ BBB)
(c/f human autism!)

PhosphoCREB- (CAMP, Calcium- gene induction)

Control (PBS)

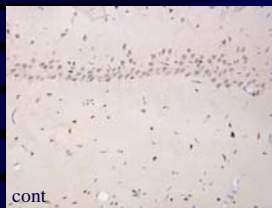


Propionate (PPA)

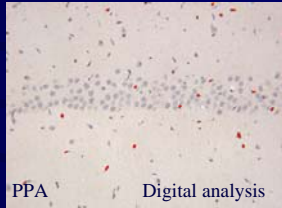


PPA can induce multiple genes implicated in learning, memory
addiction, neurodevelopment
Environment influencing genetic expression!

Is Propionate Cytotoxic? - Activated Caspase 3⁺(apoptosis)



cont



PPA

Digital analysis

Propionic acid is not grossly neurotoxic in hippocampus
Neuroinflammation with little neurotoxicity
Neuroprotectant (histone deacetylase inhibitor)

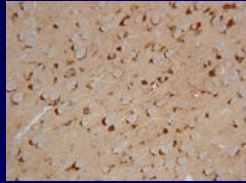
Pathology – Additional Markers Tested

Immunoglobulin G – Leaky brain or autoimmunity

Control (PBS)



Propionate (PPA)



Interleukin 6 (inflammatory cytokine)

PBS

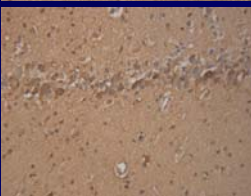


WM

PPA



Hipp



Anti Nitrotyrosine Immunoreactivity- oxidative stress

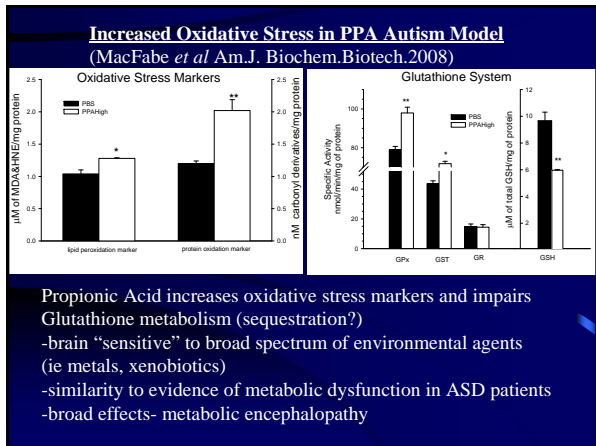


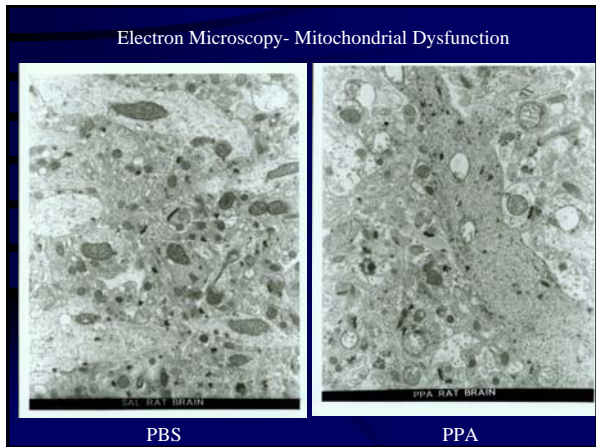
Saline



High Dose Propionate

Propionate causes increase anti Nitro-tyrosine immunoreactivity in hippocampal formation increases “oxidative stress”

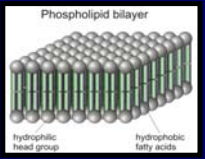
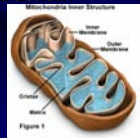
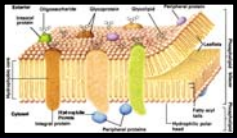




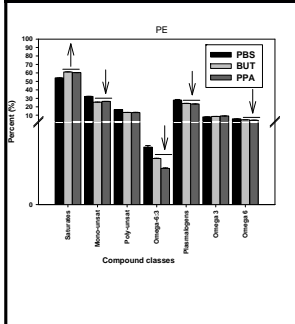
Functions of Fatty Acids

- **3 main functions:**
 - Energy storage
 - Structural components of cell membranes
 - Act as signal molecules in many metabolic processes
 - Abnormal fatty acid composition in Autism
 - Relative carnitine deficiency in ASD
 - Mitochondrial disorder?
 - (acquired?)

e.g. neuronal cell membrane

Phospholipids in autism model (Thomas et al; J. Neurochem. 2010)



I.E Phosphatidylethanolamine

Increase saturates

Decrease:

monosaturates
omega 6/3

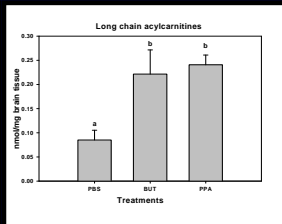
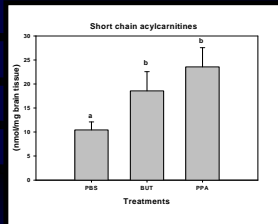
Plasmologens (antioxidant)

Same trend in

Phosphatidylcholine
Phosphatidylserine/inositol
Sphingomyelin (White matter)
Cardiolipin (mitochondria)

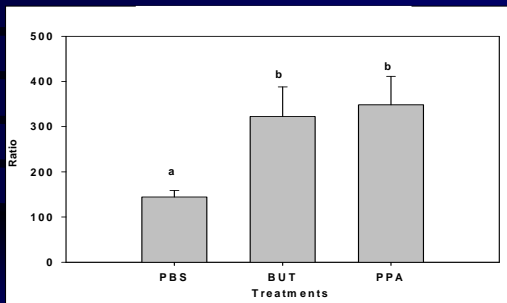
SCFA alter membrane fluidity,
Signalling, Antioxidant,
mitochondrial function

Long and short chain acylcarnitines (Thomas et al; J. Neurochem. 2010)

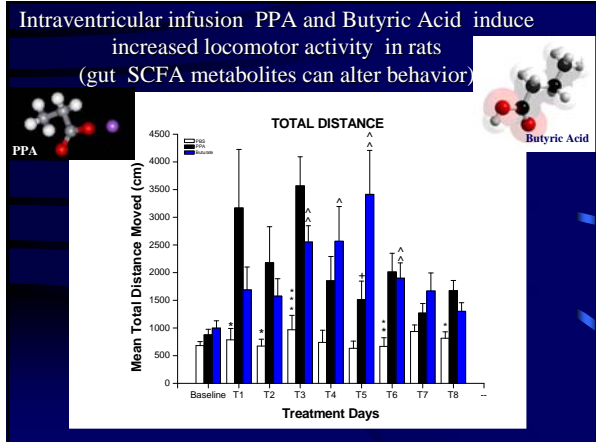


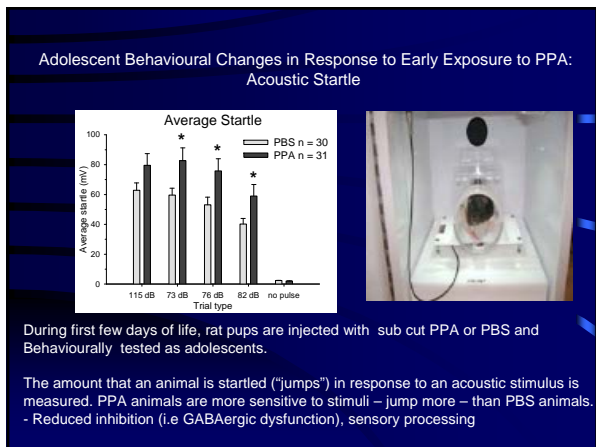
PPA causes increase in CNS Acylcarnitines
Relative Carnitine deficiency- same as ASD patients
Binds to carnitine and CoEnzyme A- mitochondrial dysfunction

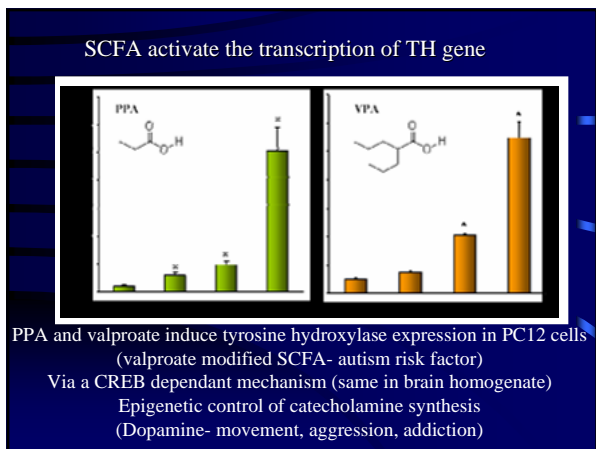
Ratio of bound to free carnitine

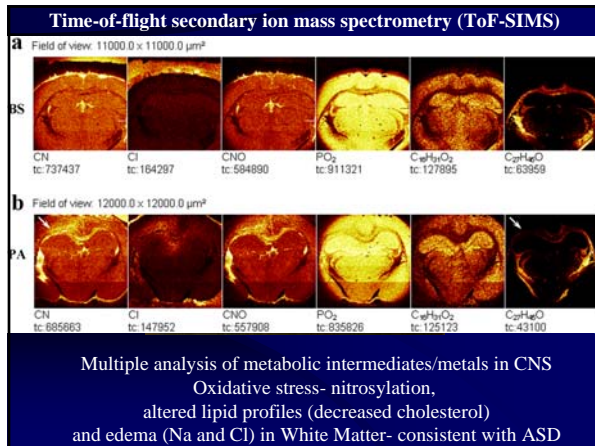


PPA causes increase in CNS bound carnitine- as in ASD patients









Diabetes **Autism**

Type I *Type II*

Can't metabolize glucose **Can't metabolize SCFAs?**

Multi- system involvement
Multiple Causes (Genes/diet/environment)
Present with Metabolic Crisis (i.e infection)
Treatment-Carbohydrate restriction (direct/indirect)
Treatment-Insulin/glyburide Carnitine/bacterial
eradication/probiotics/MB12?
Multi- system approach

Common Infections, Chronic Antibiotics, Clostridia and Carnitine= Colic, Convulsions and Compulsions "The Perfect Storm"

Carnitine


Carnitine- Shuttle for mitochondrial fatty acid beta oxidation
 Routine pre- peri or post natal infections-
 Pochini'08 Long term antibiotics (beta lactams)-deplete carnitine transport
 "Barren Gut"->Growth of clostridials- increased SCFA production
 Further sequestration of carnitine
 Impaired fatty acid metabolism- mitochondrial encephalopathy

Sooo...Is our PPA rodent model like human autism?

- Hyperactivity/ Complex Movement/Object fixation
- Intermittent seizure
- Subcortical spiking with movement
- Kindling/ Neuroplasticity
- Social Impairment
- No gross neurotoxicity
- Astrogliosis/microglia/ Neuroinflammation
- White matter damage (lipoperoxidation, edema, cholesterol)
- Oxidative stress/ impaired glutathione (broad spectrum detoxifier)
- Relative carnitine deficiency/increased acylcarnitines/ altered phospholipids
- Induce catecholamine/CREB expression (epigenetics)

•Propionic acid is known to cause:

- Neutrophil/monocyte migration (specific SCFA receptors)
- Mitochondrial uncoupling (fatty acids), increases in odd chain FAs, low chol.
- Neuronal structural changes (cytoskeleton)/gene expression
- Intracellular acidification - Dopamine/glutamate/5HT release – gene induction
- Impairment in cell-cell signal transduction (gap junctions, cytokines)



ARE WE THERE YET !?!



Cautious Optimism- need for further rational study!
Short Chain Fatty Acids not "Good" or "Bad"
Timing/Amount/Genetic Sensitivity Critical

Summary

Autism is a complex problem needing a multi-disciplinary approach with modern brain research techniques, much is available to rationally examine autism as a defined brain disorder

Factors in brain development- neural migration, embryonic cell death
toxic environmental compounds (dietary and enteric fatty acids)
role of diet and gut bacteria (antibiotic exposure)

Gut metabolites can alter brain electrical activity, behaviour,
Pathology, gene induction and cellular metabolism (mitochondria)

Screening, lipid profile/carnitine/acylcarnitine-microflora

Carbohydrate restriction, carnitine/omega 3/MB12/bacterial eradication
Variable exposure/breakdown in humans/antibiotics/microflorae

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AUTISM- HOPE FOR THE FUTURE