A sunset over a large body of water, likely a lake or bay. The sky is a mix of blue, orange, and yellow. The water reflects the colors of the sky. In the foreground, several large rocks are visible, with silhouettes of people sitting or standing on them. The overall scene is peaceful and scenic.

# The Enteric (Gut) Microbiome Modulates Mitochondrial Function

**Richard E. Frye, M.D., Ph.D.**

Child and Behavioral Neurologist

Arkansas Children's Hospital

University of Arkansas for Medical Sciences

## Talking Points

The enteric (gut) microbiome has an important influence in on health and disease states in humans.

The enteric microbiome influences the human host using chemical mediators, some of which can directly effect mitochondrial function

Short chain fatty acids produced by gut bacteria not only modulate mitochondrial function and cellular regulatory pathways, but can also be used as mitochondrial fuels.



## THE HUMAN

Bacteria, fungi, and viruses outnumber human cells in the body by a factor of 10 to one. The microbes synthesize key nutrients, fend off pathogens and impact everything from weight gain to perhaps even brain development. The Human Microbiome Project is doing a census of the microbes and sequencing the genomes of many. The total body count is not in but it's believed over 1,000 different species live in and on the body.

**25 SPECIES**

in the **stomach** include:

- *Helicobacter pylori*
- *Streptococcus thermophilus*

**500-1,000 SPECIES**

in the **intestines** include:

- *Lactobacillus casei*
- *Lactobacillus reuteri*
- *Lactobacillus gasseri*
- *Escherichia coli*
- *Bacteroides fragilis*
- *Bacteroides thetaiotaomicron*
- *Lactobacillus rhamnosus*
- *Clostridium difficile*

## MICROBIOME

**600+ SPECIES**

in the **mouth, pharynx and respiratory system** include:

- *Streptococcus viridans*
- *Neisseria sicca*
- *Candida albicans*
- *Streptococcus salivarius*

**1,000 SPECIES**

in the **skin** include:

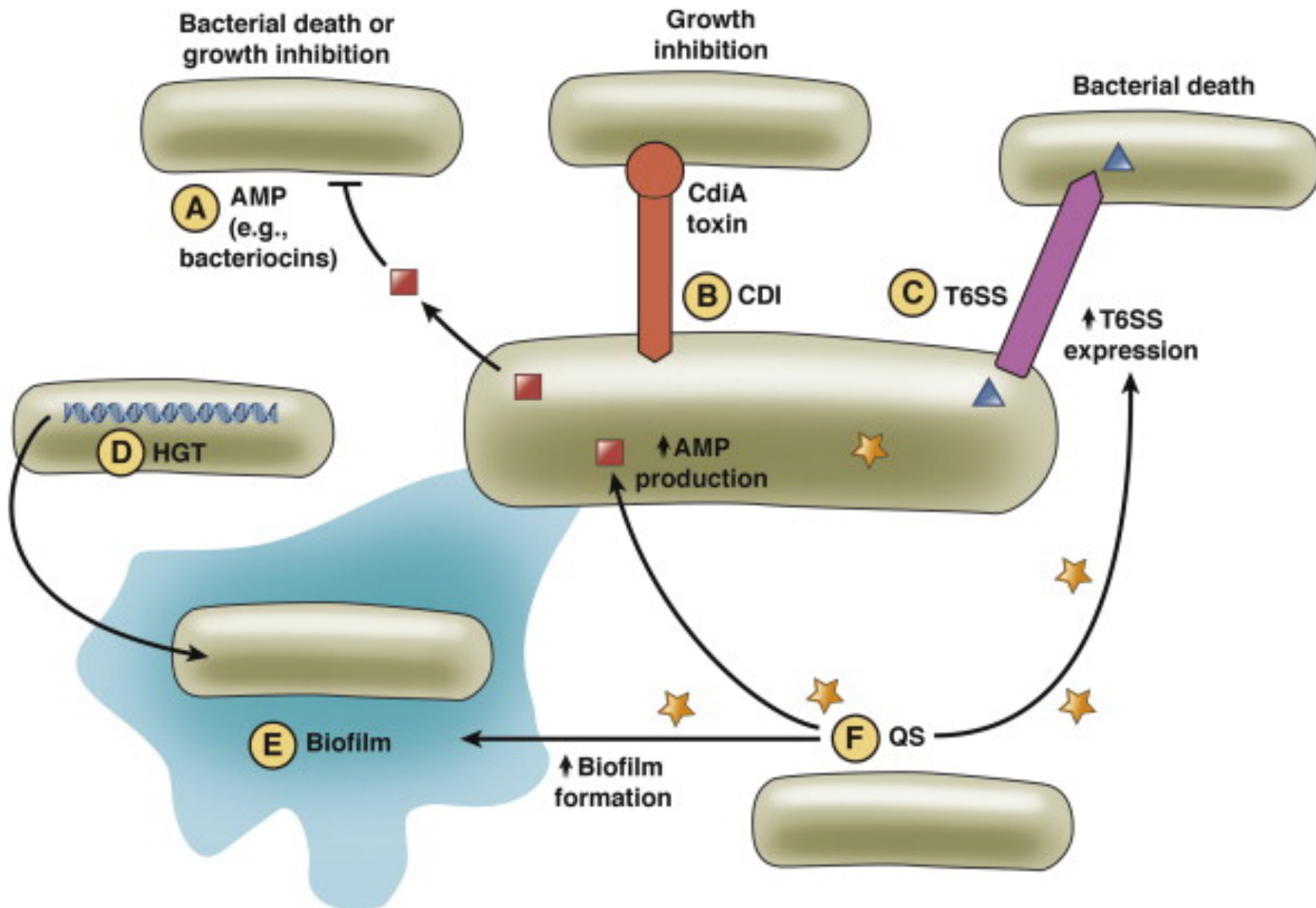
- *Pityrosporum ovale*
- *Staphylococcus epidermidis*
- *Corynebacterium jeikeium*
- *Trichosporon*
- *Staphylococcus haemolyticus*

**60 SPECIES**

in the **urogenital tract** include:

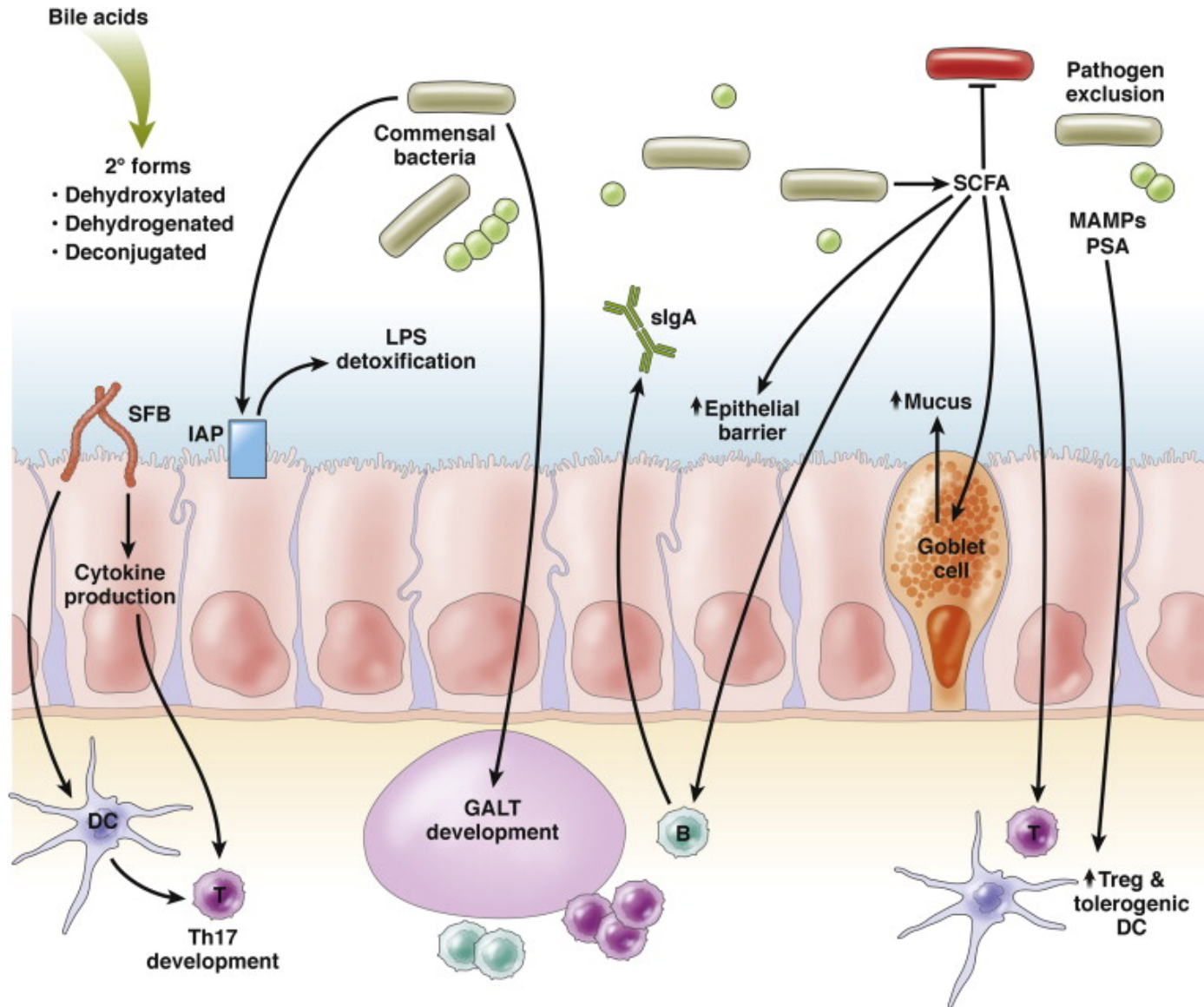
- *Ureaplasma parvum*
- *Corynebacterium aurimucosum*

Bacteria use a complex communication network to thrive in an environment

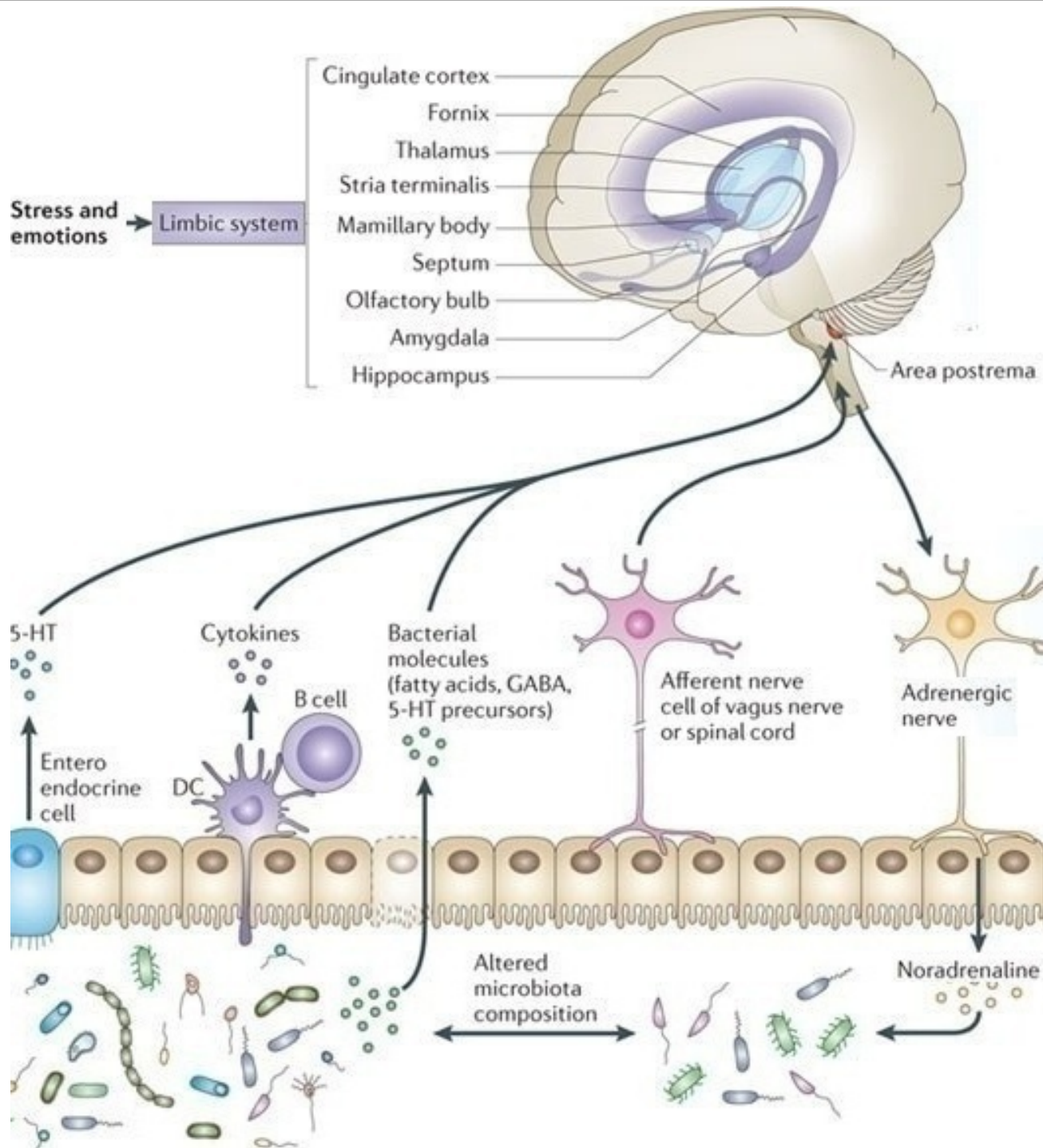




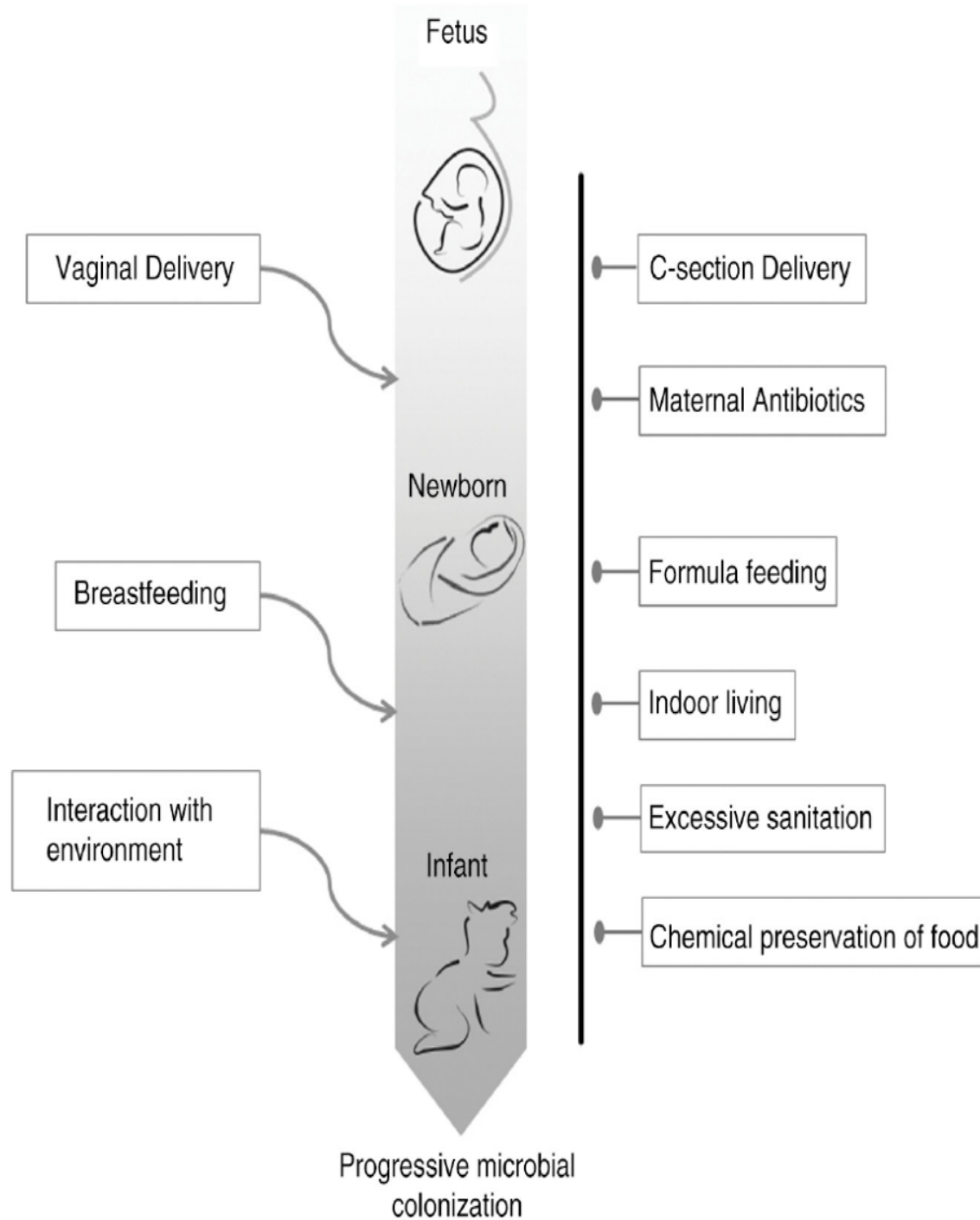
## The microbiota induces host immune tolerance

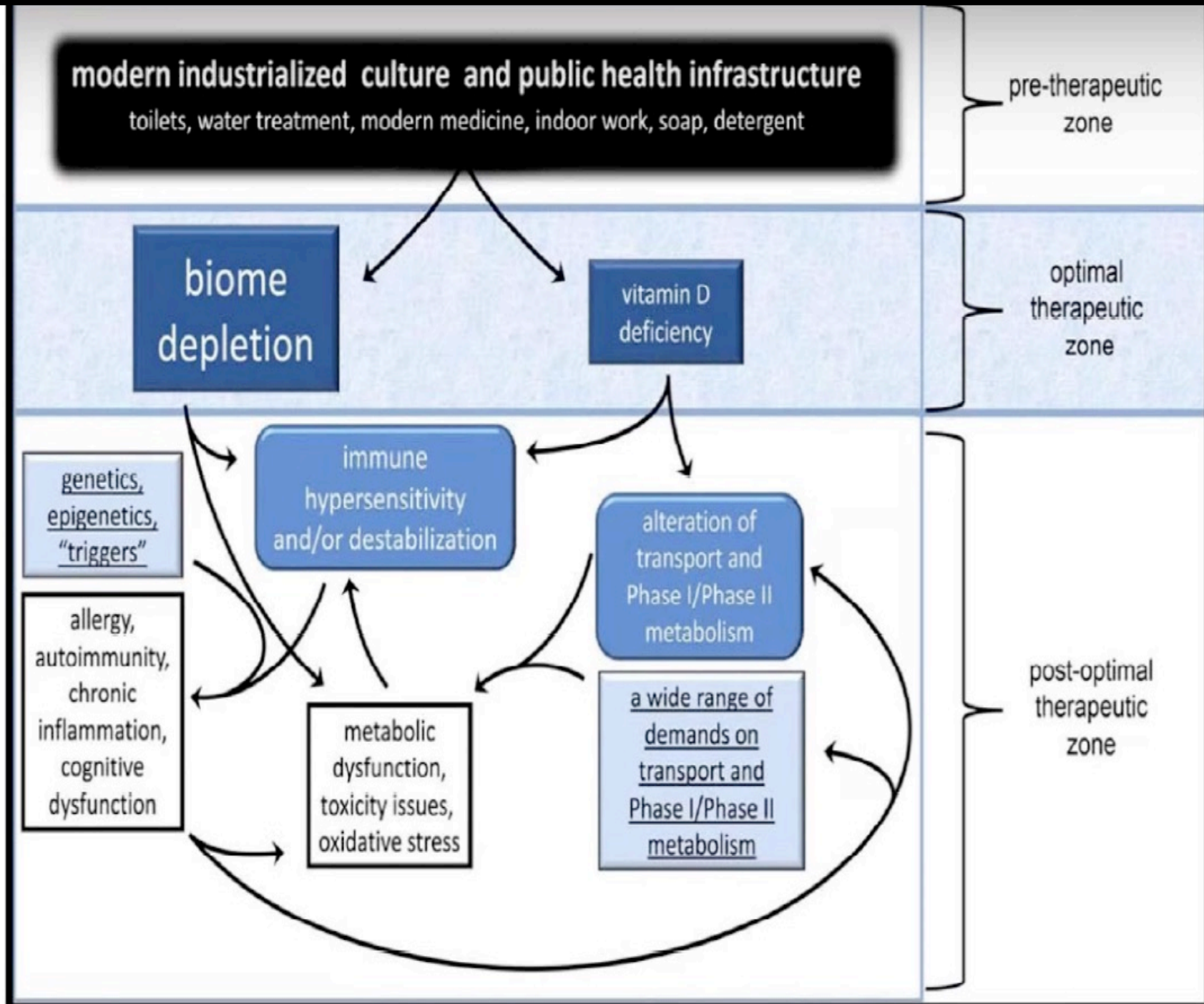


Segmented Filamentous Bacteria  
Gut associated lymphoid tissue

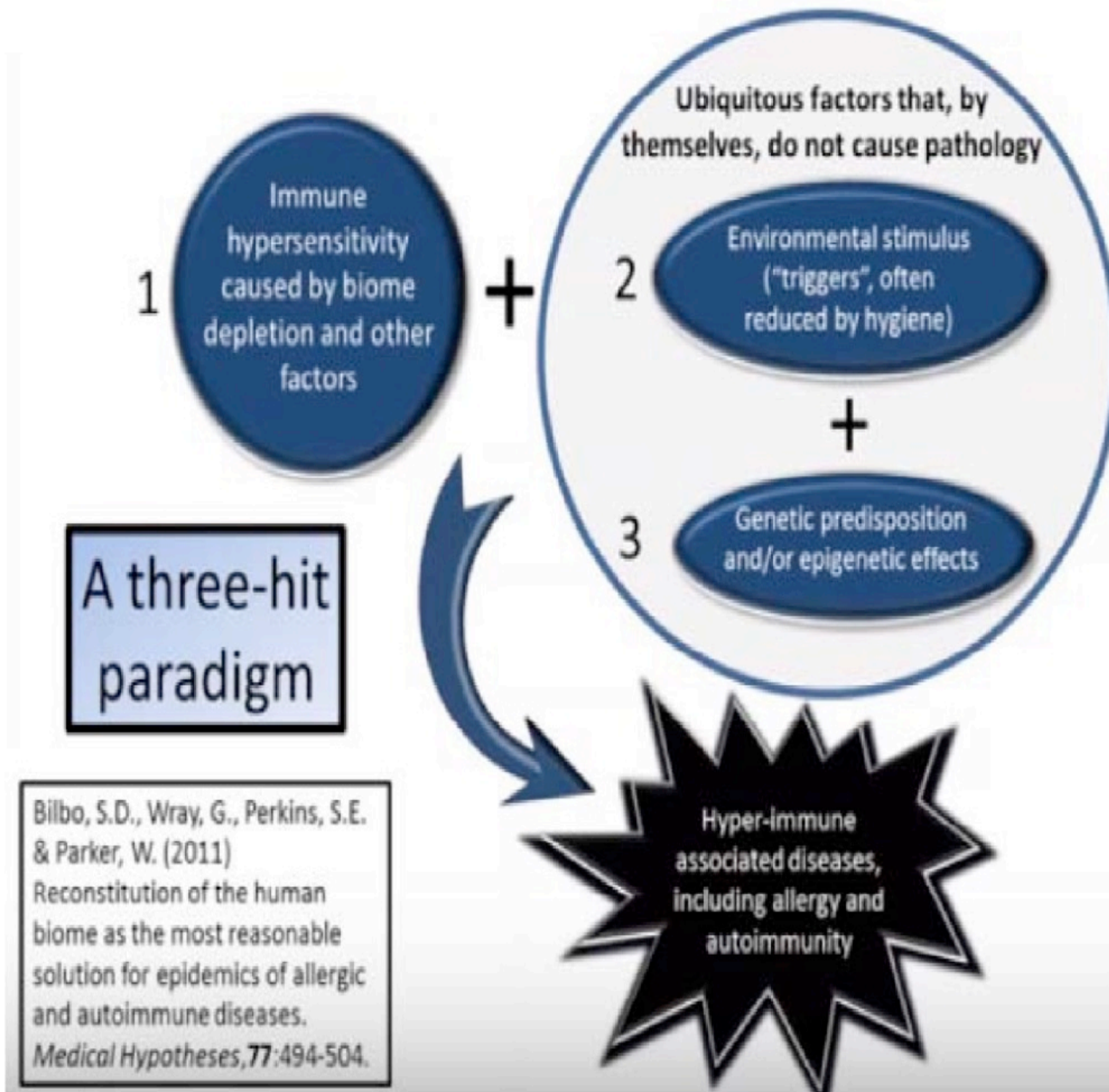


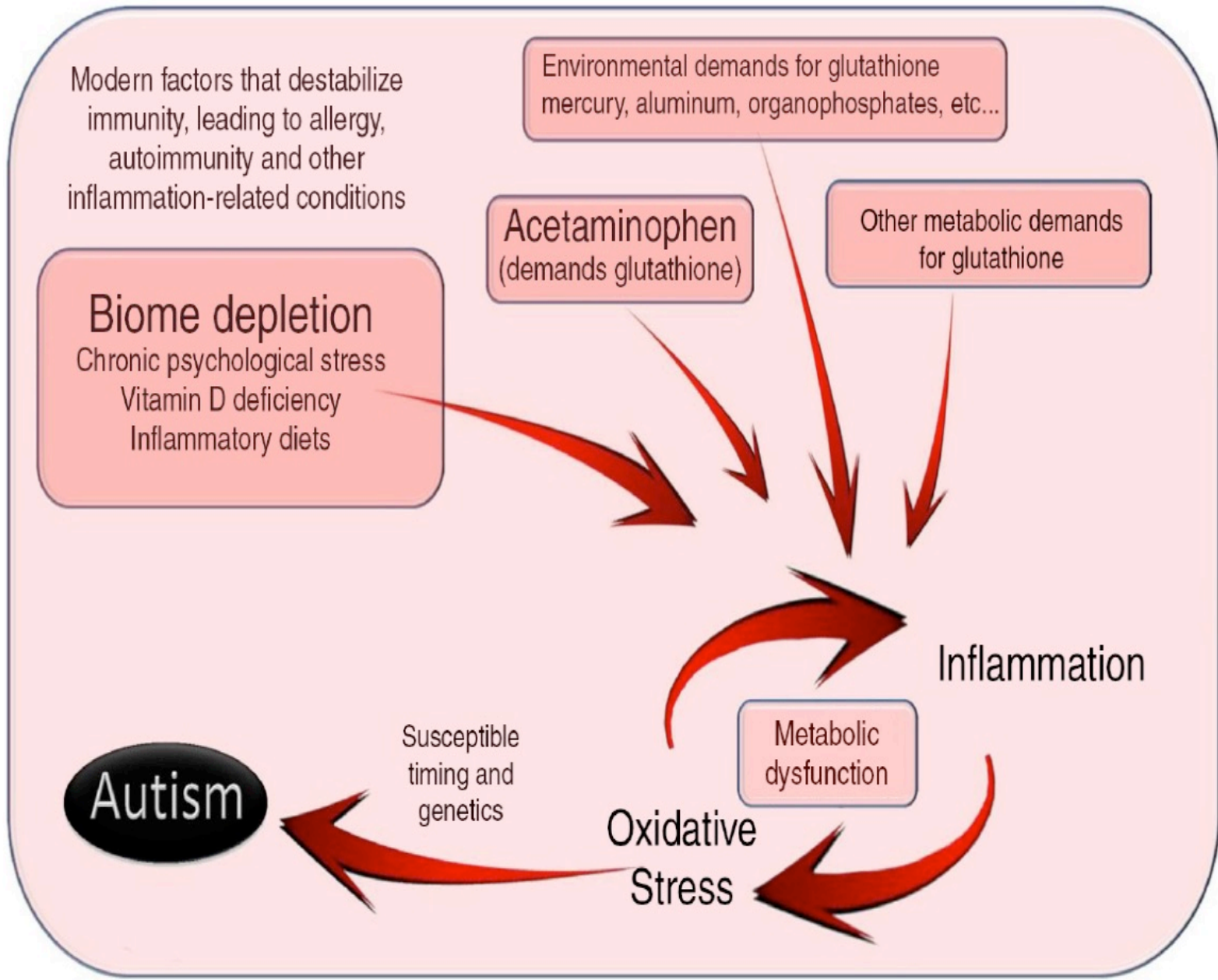




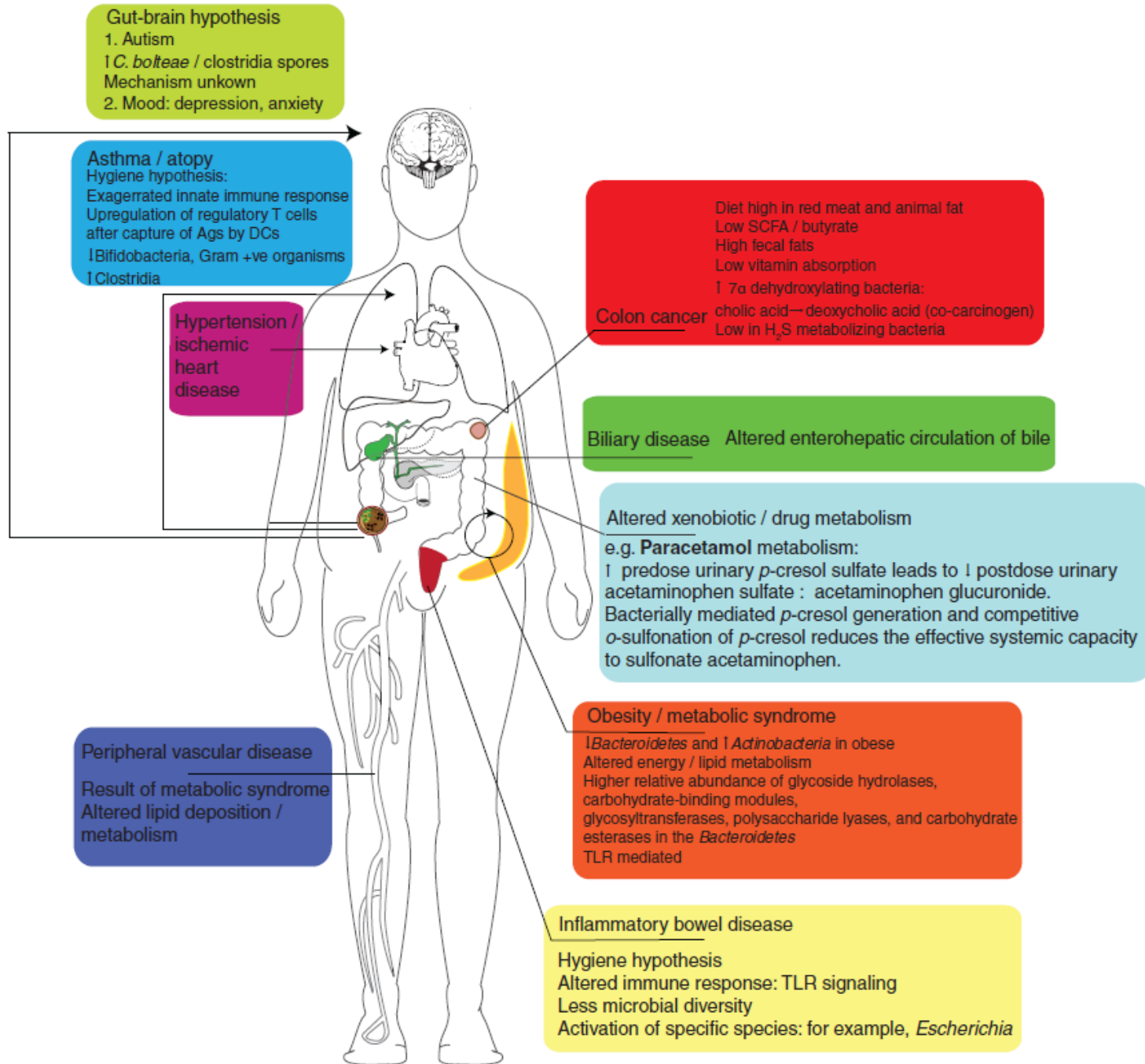












## The Significance of the Enteric Microbiome on the Development of Childhood Disease: A Review of Prebiotic and Probiotic Therapies in Disorders of Childhood

John Slattery<sup>1,2</sup>, Derrick F. MacFabe<sup>3</sup> and Richard E. Frye<sup>1,2</sup>

**Table 1.** Microbiome disruption by condition summary.

CONDITION	RELEVANT FINDINGS
Prematurity	↑ Proteobacteria ↓ Microbial diversity
Necrotizing enterocolitis	Blooms of <i>Proteobacteria</i> prior to disease onset
Sepsis	Altered microbiota structure and composition prior to disease onset has been reported, but specific microbiota reported is inconsistent across studies
Colic	Decreased microbial diversity and increased anaerobic bacteria
Malnutrition	Anaerobic depletion, early dysbiosis, and intestinal pathogenic overabundance with decreased bacterial diversity
Eczema	Early colonization with opportunistic species may be important in disease initiation

**Table 1.** Microbiome disruption by condition summary.

CONDITION	RELEVANT FINDINGS
Allergies	↓ Species diversity
Asthma	No clear pattern
Inflammatory bowel disease	Data is sparse, no consistent pattern
Type I diabetes	↑ <i>Bacteroidetes:Firmicutes</i> ratios, ↑ <i>Clostridia</i> species ↓ Butyrate-producing bacteria ↓ Bacterial diversity ↓ Community stability Alterations in the microbiome seem to precede disease onset
Type II diabetes and obesity	↑ <i>Firmicutes:Bacteroidetes</i> ratio ↑ SCFAs
Autism spectrum disorder	↑ <i>Clostridial</i> species ↑ <i>Suttetrella</i> and <i>Desulfovibrio</i> species



## Enteric Ecosystem Disruption in Autism Spectrum Disorder: Can the Microbiota and Macrobiota be Restored? John Slattery<sup>a,b</sup>, Derrick F. MacFabe<sup>c</sup>, Stephen G. Kahler<sup>a,d</sup> and Richard E. Frye<sup>a,b</sup>

Potential Trigger	Possible Remediation Plan
Mitochondrial Toxins	Avoid and Eliminate [ see 4a and 4b] If certain medications can't be avoided a mitochondrial cocktail such as carnitine, Co-Q10, B Vitamins, Creatine Monohydrate, and antioxidants [49]
Antibiotics (Abx)	Provide Pre/Probiotics with Abx and/or NAC. Supplement with carnitine if using $\beta$ -Lactams
Acetaminophen	Avoid and Eliminate. Pre-treat with NAC if unavoidable.
Poor Folate Metabolism and/or Absorption	Treat with reduced folates (e.g. folic acid) and avoid folic acid
Bovine Milk Products	Eliminate or Minimize Use
Maternal Infection	Minimize exposures and medications that could further complicate development
Maternal Autoimmune Reaction	Pre/Probiotic Supplementation and other immune supporting agents
Psychological Stress Management	Meditation, Yoga, and/or other relaxation techniques to mothers during pregnancy.

Potential Trigger	Possible Remediation Plan
Pre/postnatal Toxins	Avoid air pollution, solvents, polychlorinated biphenyls (PCBs), phthalates, bisphenol A, and mercury exposure as well as cigarette smoking, illicit drug use, and alcohol exposure
Poor Diet	Eat foods high in microbiota accessible carbohydrates along with fruits and vegetables. FMTs in the future may be warranted
Premature Weaning and/or Formula feeding	Breastfeed for at least 6 months and/or supply breast milk from donors over formula feeding or pasteurized milk
Vitamin D and/or Tryptophan Metabolism Deficiency or Disorders	Supplement with Tryptophan and Vitamin D
Poor Maternal Sleep Hygiene	Introduction of Sleep Protocols to decrease sleep associated complications
C-Sections	Avoid elective C-Sections and reserve for emergency situations only
Helminths	Re-introduction of helminthes into the intestinal ecosystem is necessary to re-establish the balance of the ecosystem





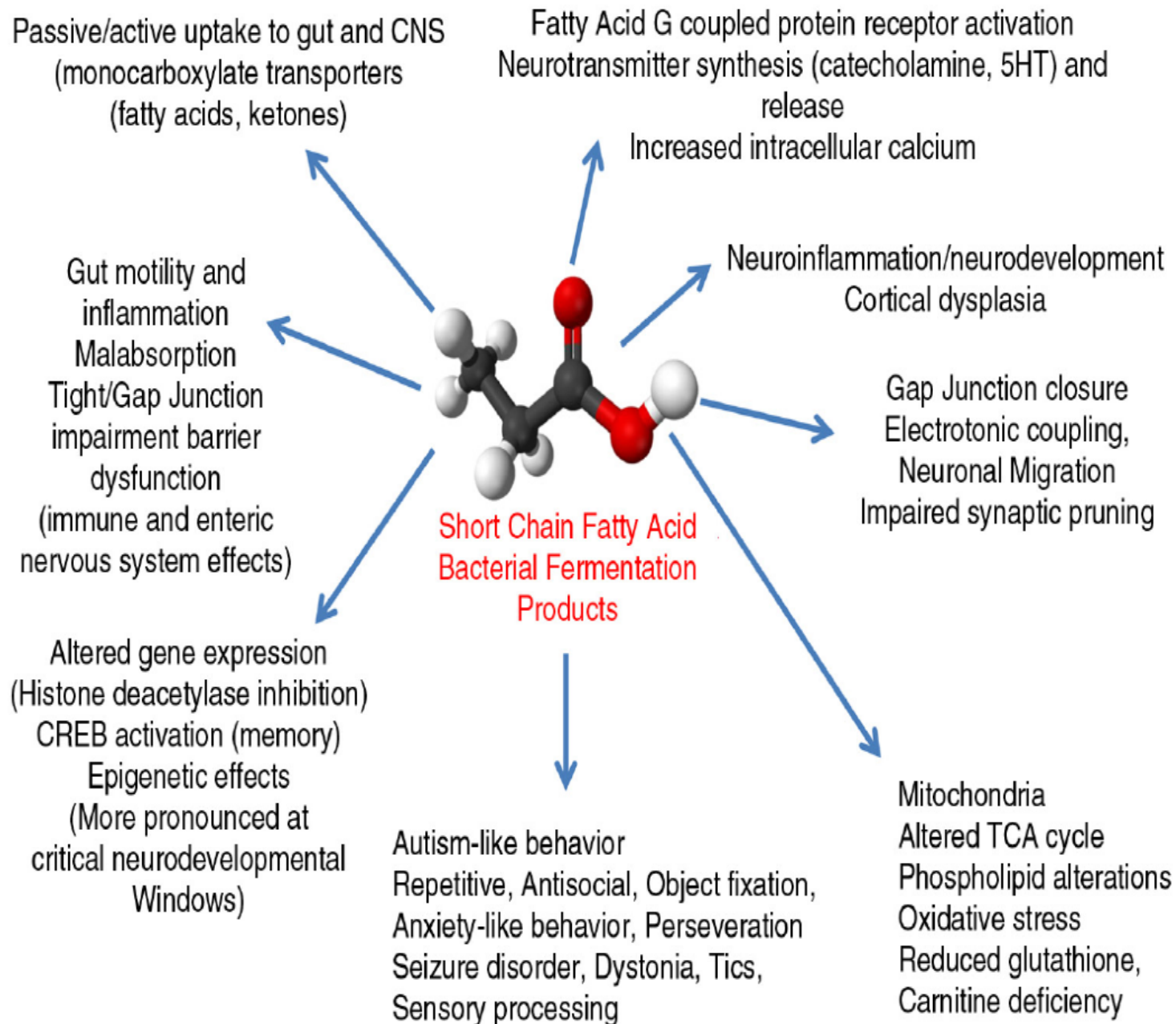
# Autism Spectrum Disorder and the Microbiome



# Propionic Acid

Model of Autism:

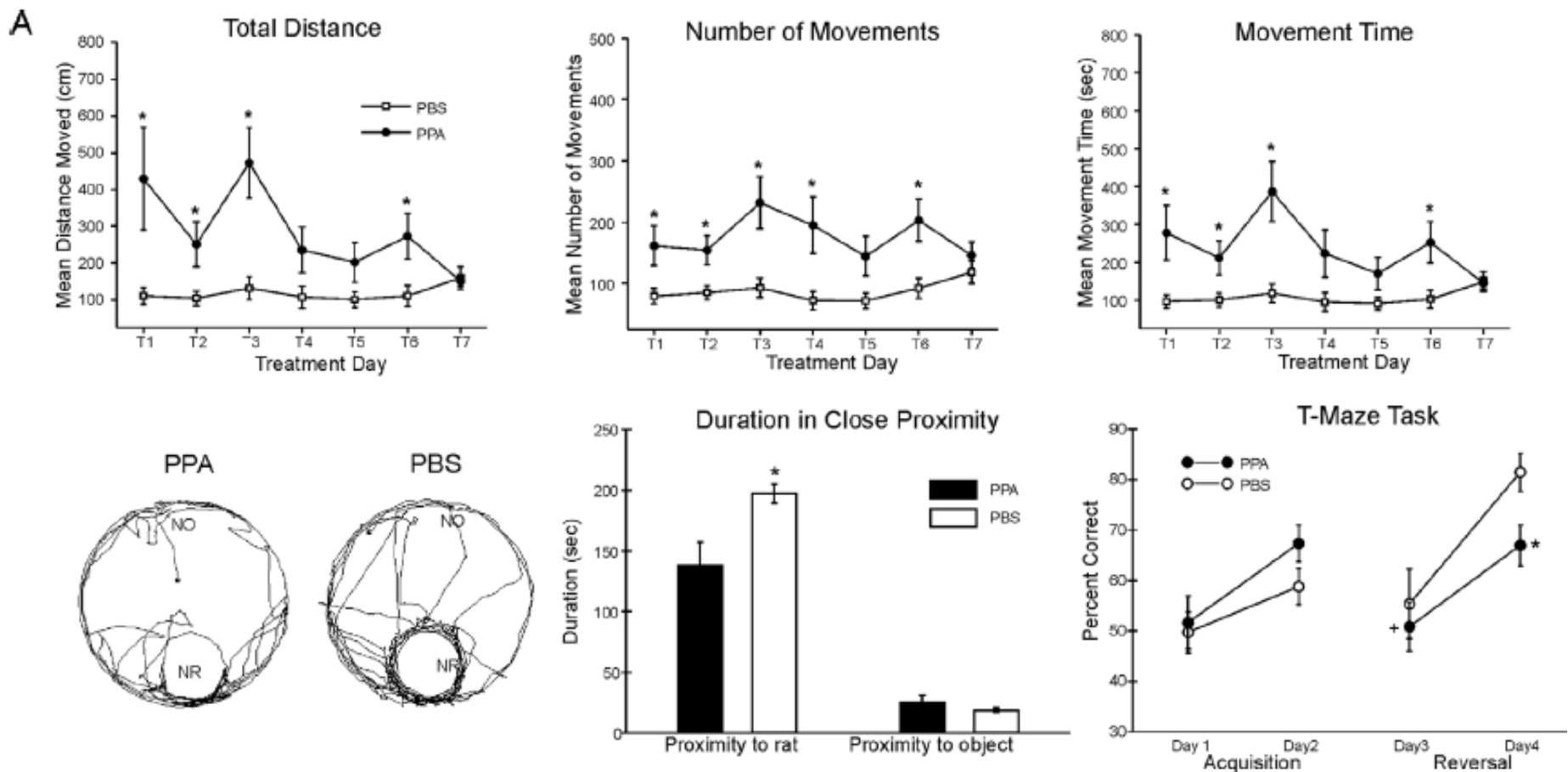
Animal Models



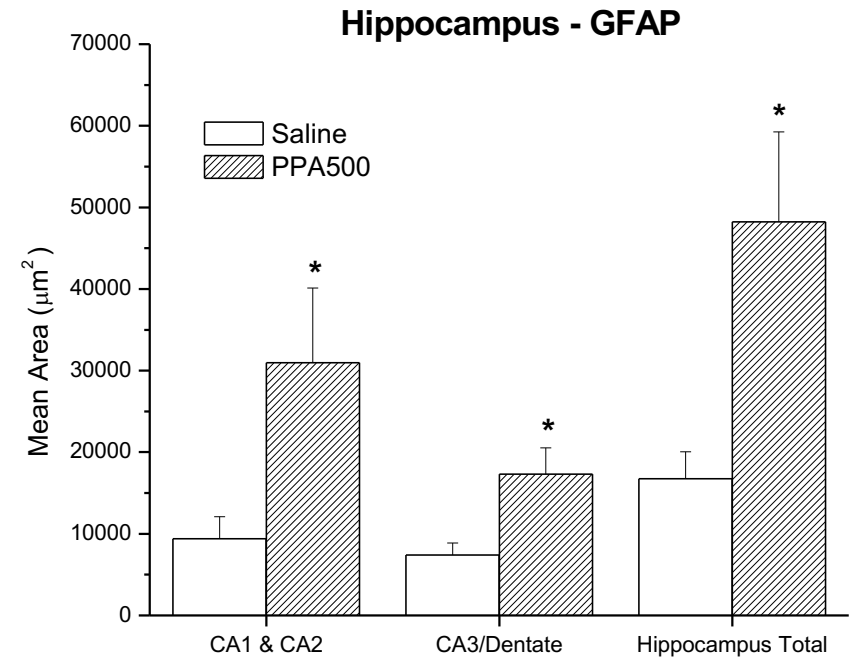
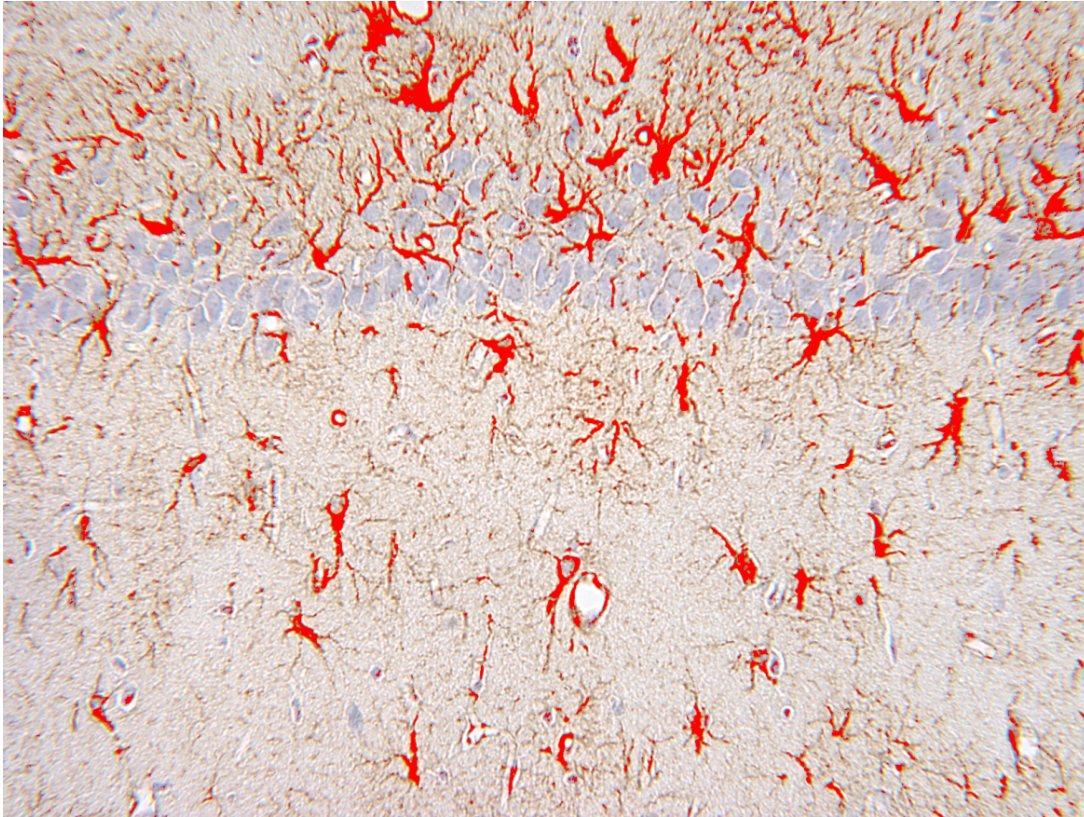


## Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders

Derrick F. MacFabe, MD\*



## Neuropathology Quantification

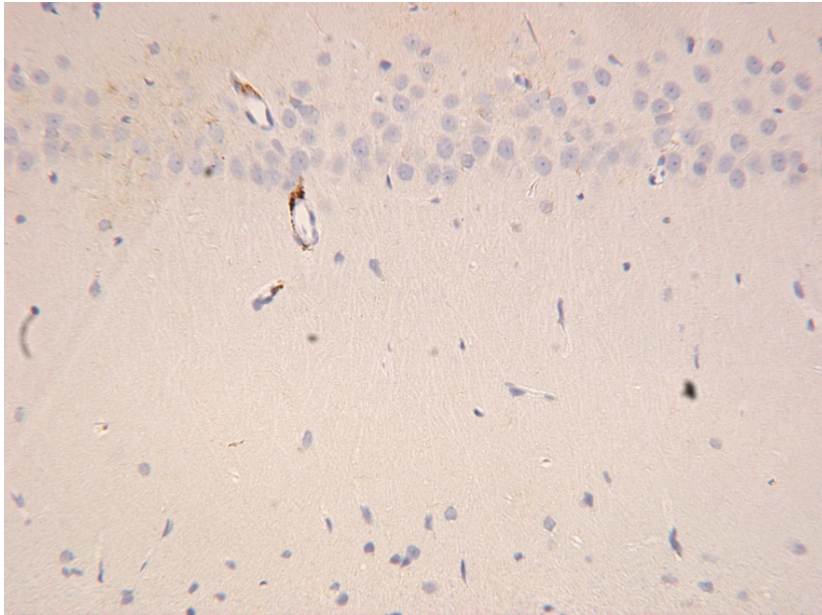


Propionate increases GFAP, Marker of Reactive Astrocytes and immunoreactivity in hippocampus

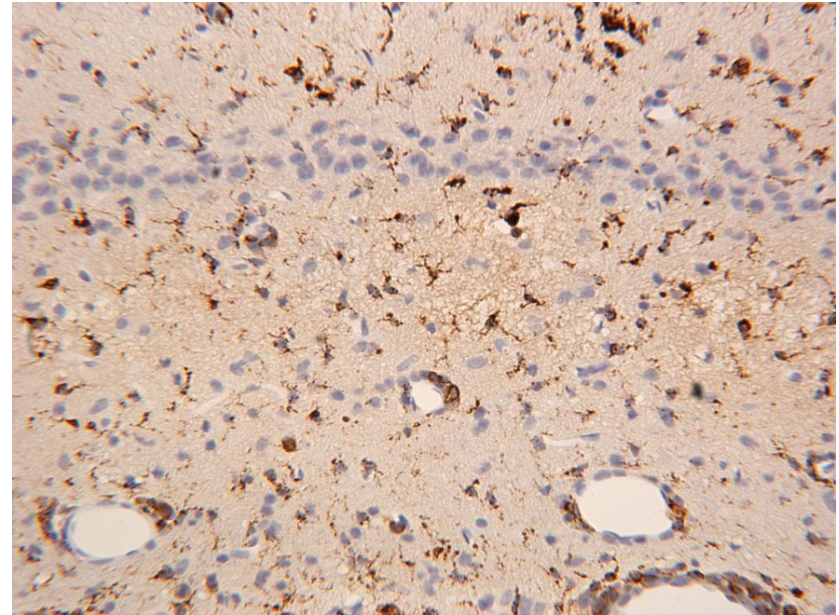


# CD68 marker of Microglia

**Control (PBS)**



**PPA**



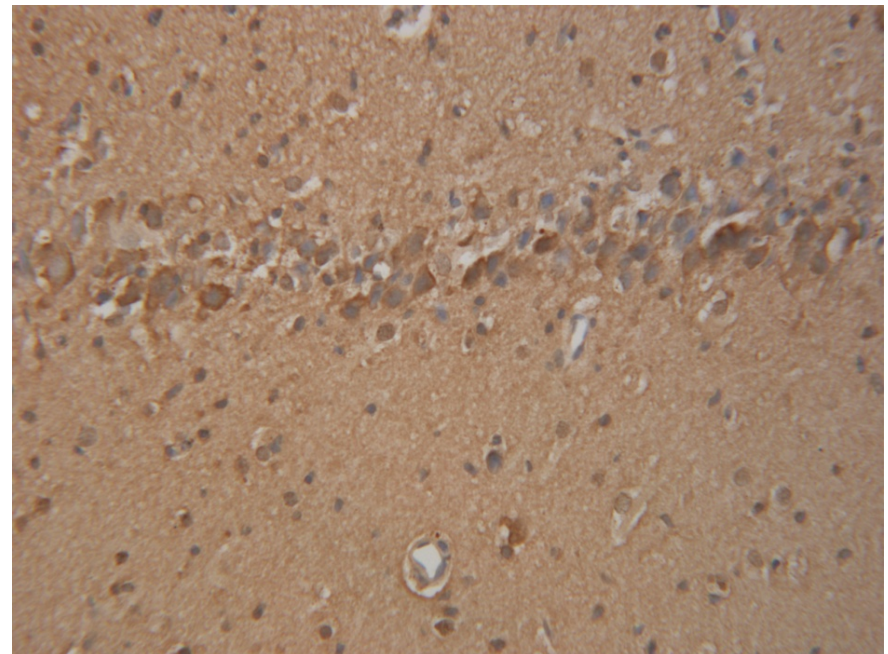
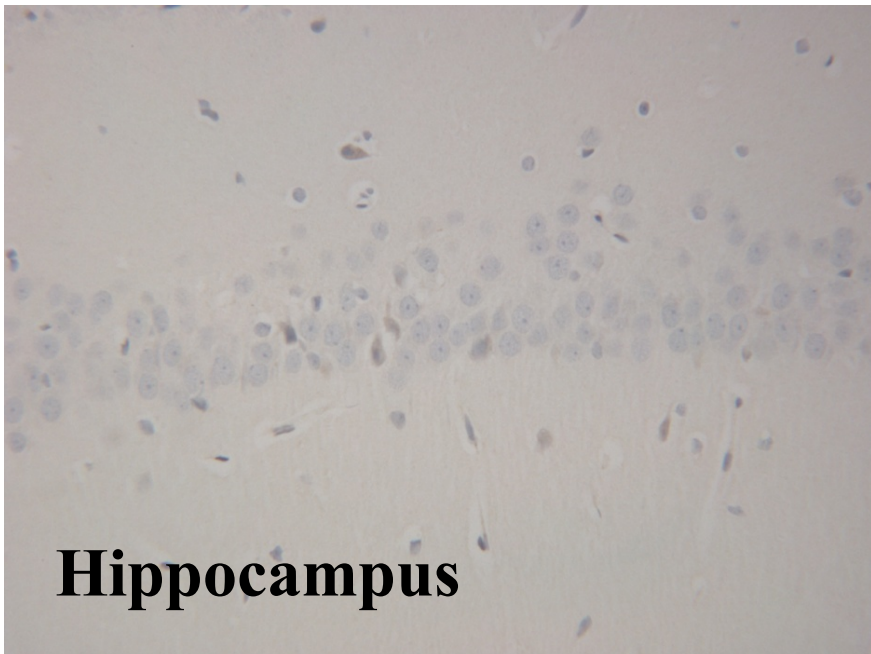
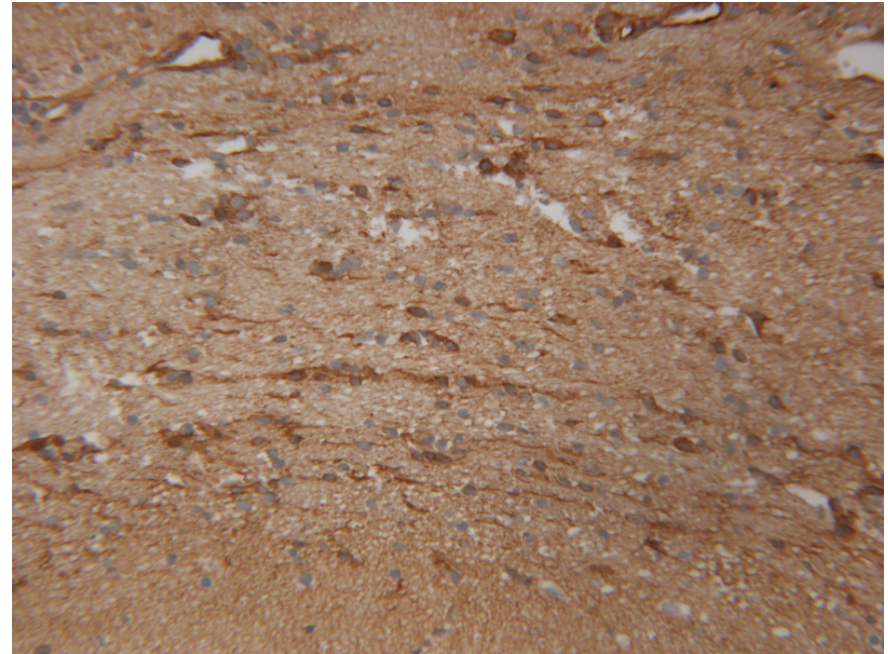
PPA increases activated microglia (neuroinflammation)



**PBS**

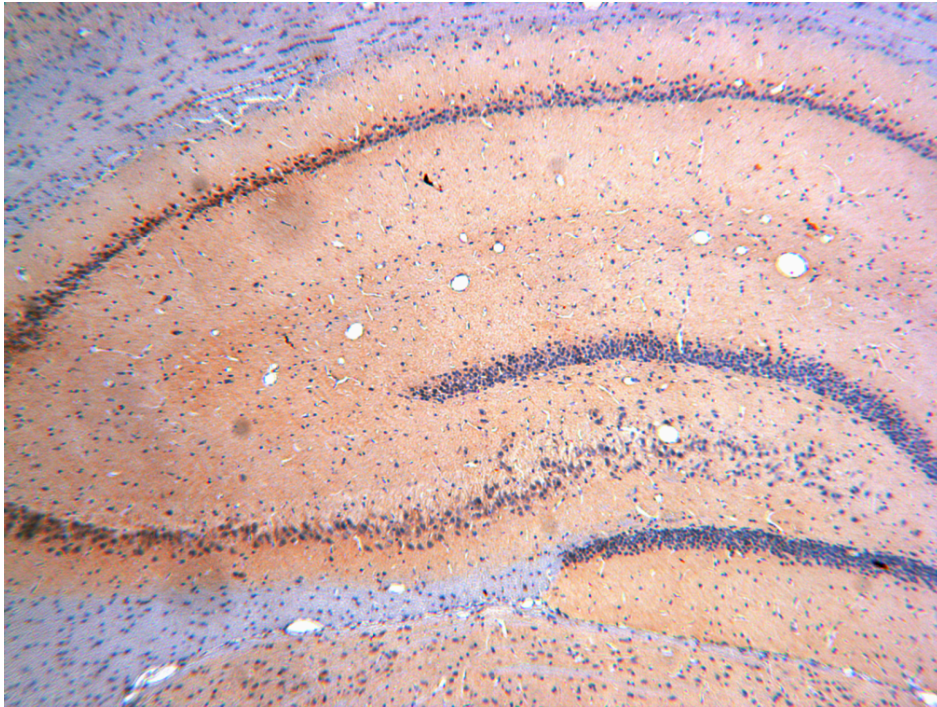
**Interleukin 6**

**PPA**





## Nitrotyrosine Immunoreactivity, a measure of oxidative stress



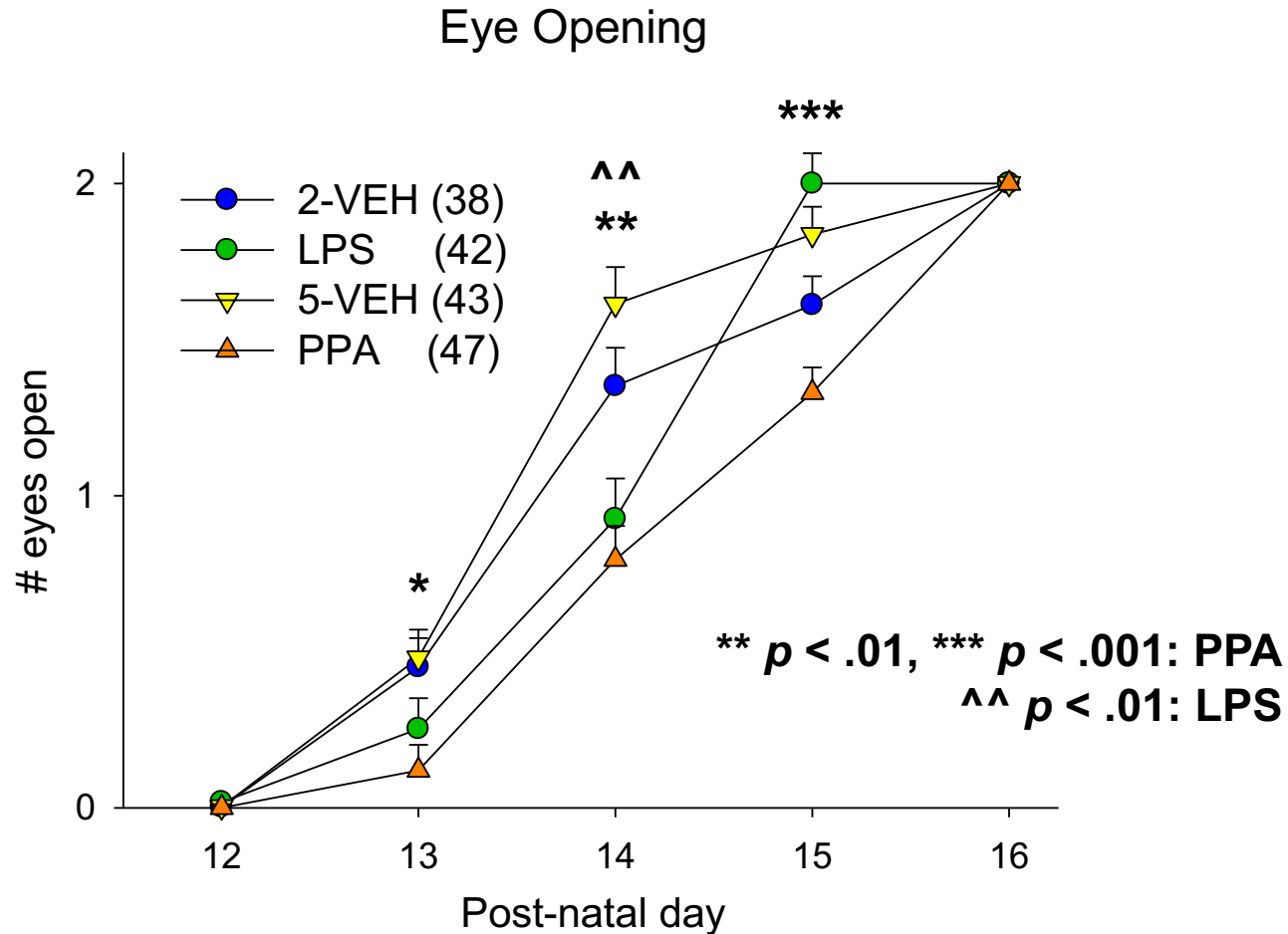
**PBS Vehicle**



**High Dose PPA**

**PPA causes increase anti Nitro-tyrosine immunoreactivity in hippocampal formation, a measure of oxidative stress**

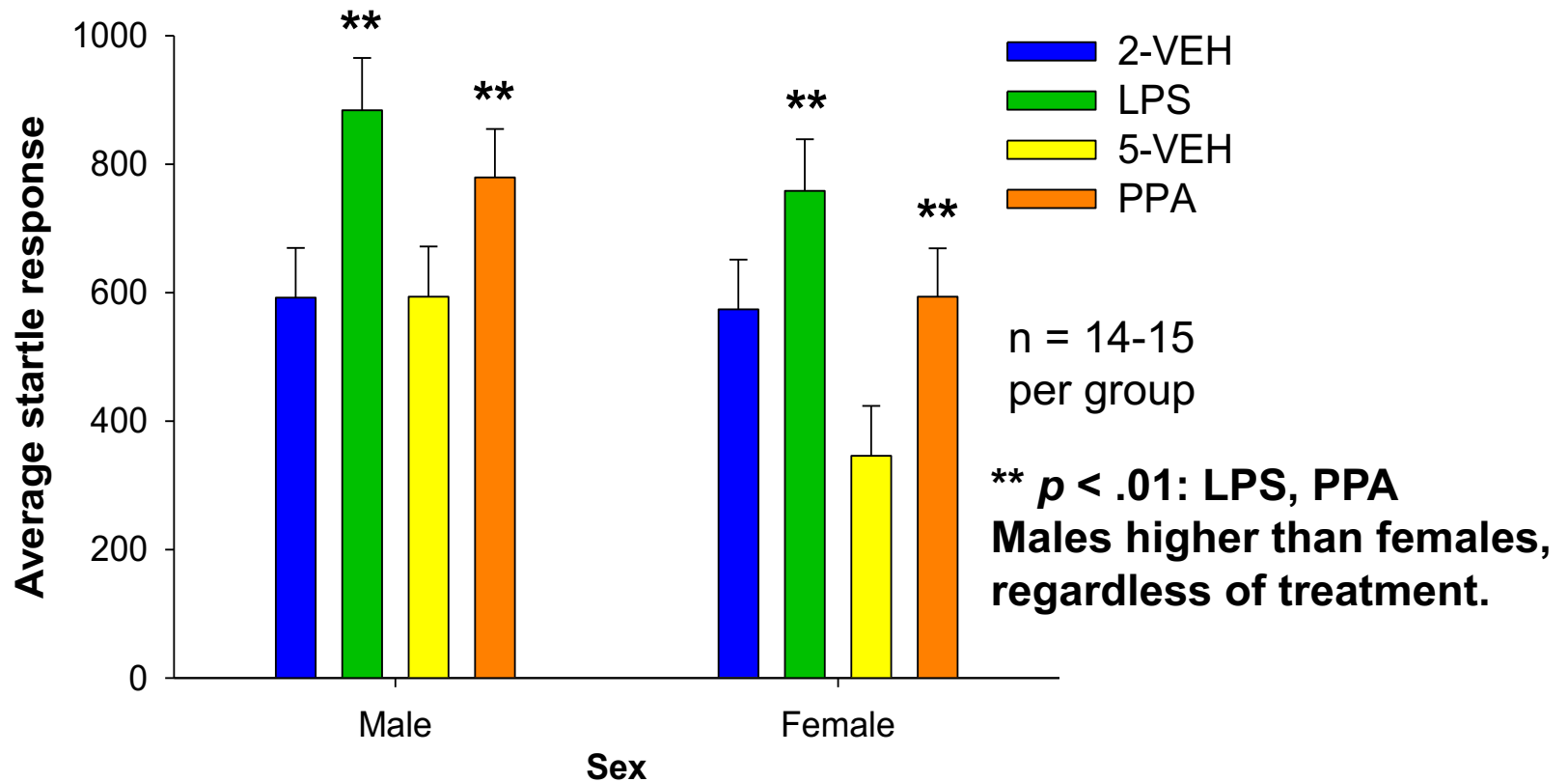
## Developmental delay in pups



**Developmental milestones are delayed in pups prenatally exposed to PPA and LPS (ie. pinna detachment, incisor eruption). (PPA similar to valproic acid- mitochondrial function/gene expression)**



## Acoustic startle response: Prenatal PPA and LPS produce hyper-sensitivity to stimulus



**Other anxiety-like behaviour also present in PPA and LPS animals.**



# Propionic Acid

Model of Autism:

Parallels of Children with Autism

Citation: *Transl Psychiatry* (2013) 3, e220; doi:10.1038/tp.2012.143  
© 2013 Macmillan Publishers Limited All rights reserved 2158-3188/13

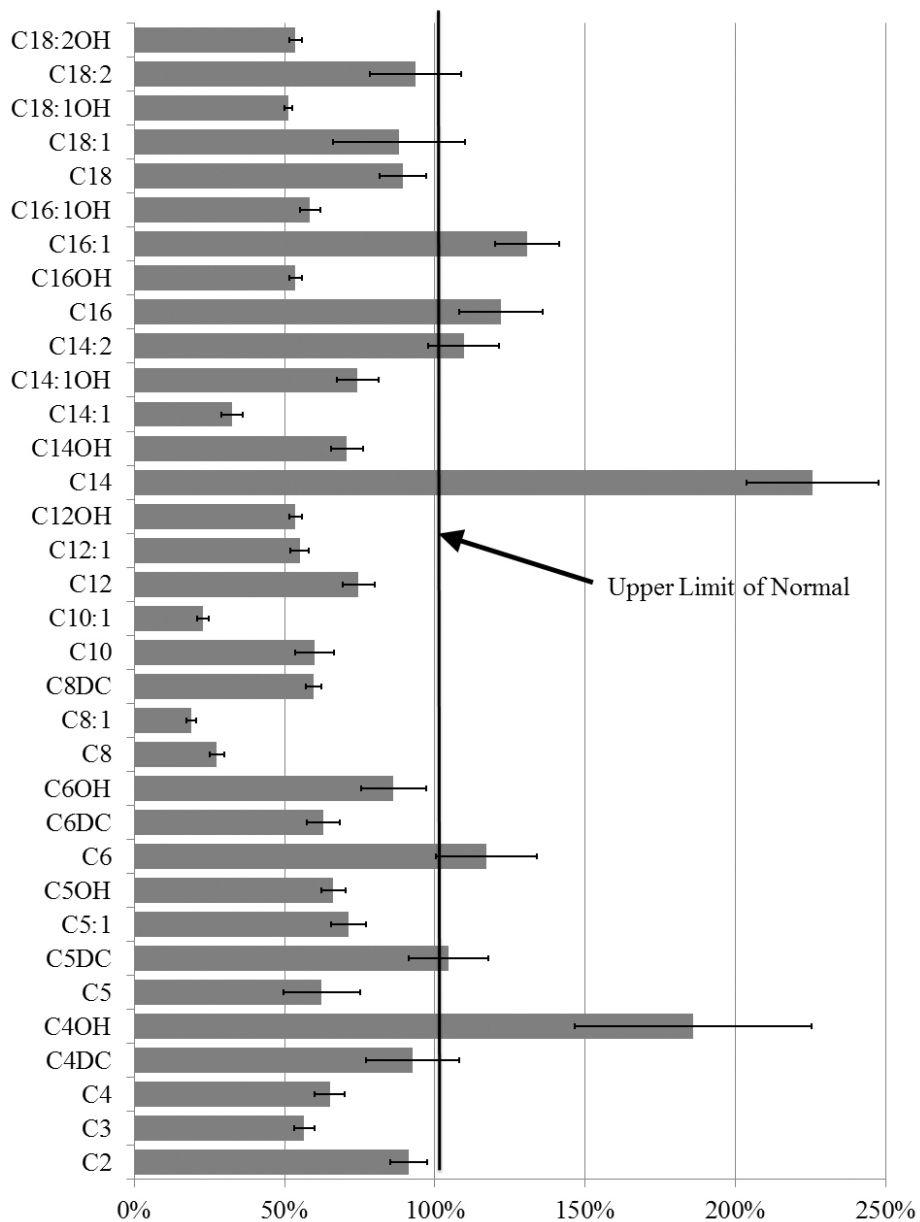
[www.nature.com/tp](http://www.nature.com/tp)

## Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder

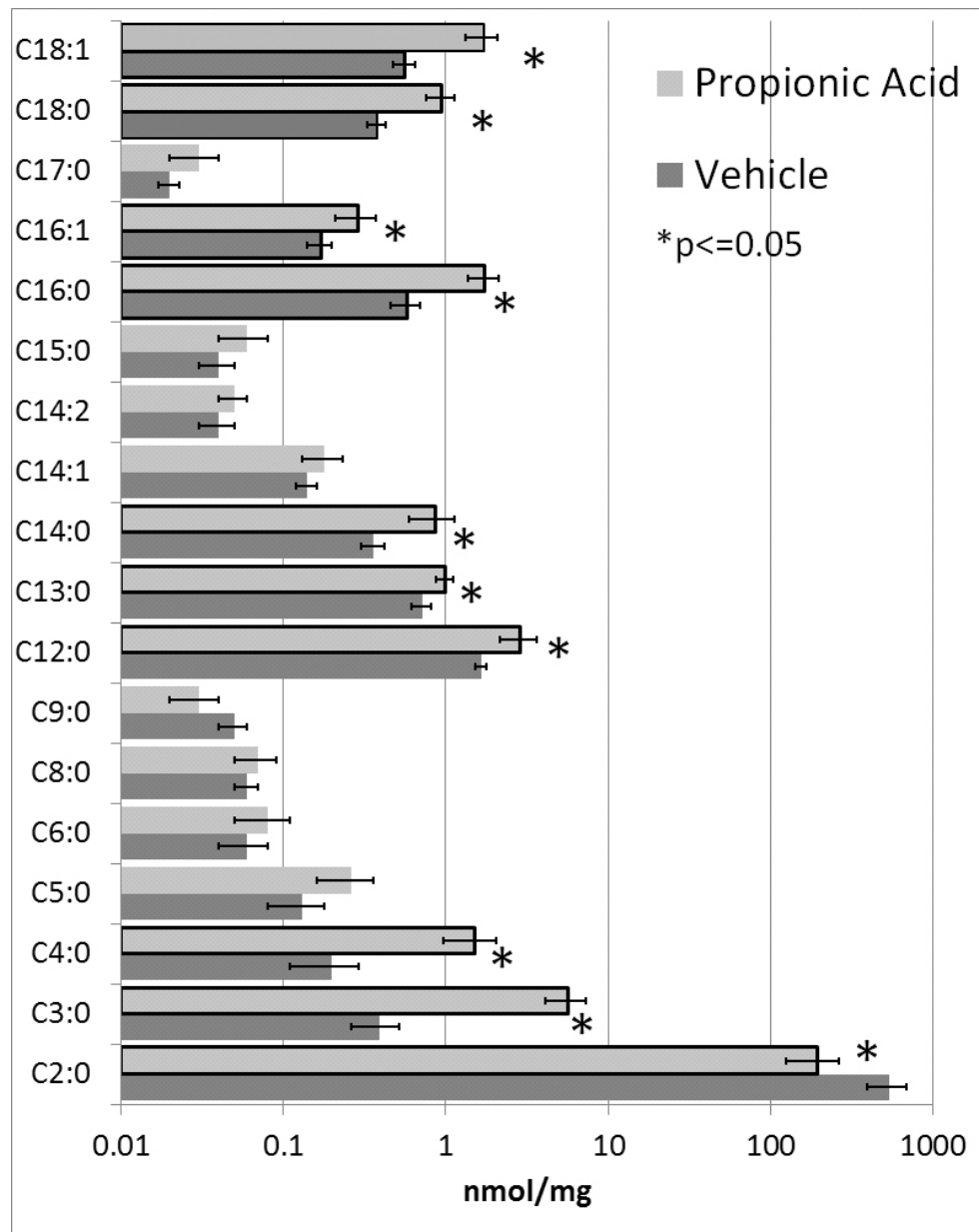
RE Frye<sup>1</sup>, S Melnyk<sup>1</sup> and DF MacFabe<sup>2</sup>

- 213 ASD patients screened with acyl-carnitine biomarkers
- 74 (35%) with  $\geq 3$  fasting acyl-carnitine elevations
- Acyl-carnitine abnormalities were confirmed in 48%
- Corrected prevalence of 17% of ASD children screened.

## Autistic Children

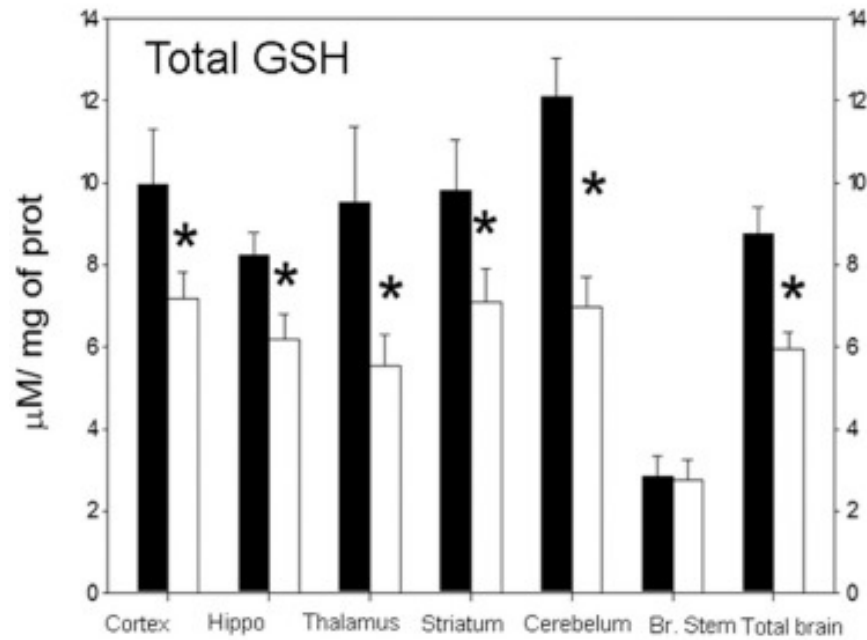


## Rodents

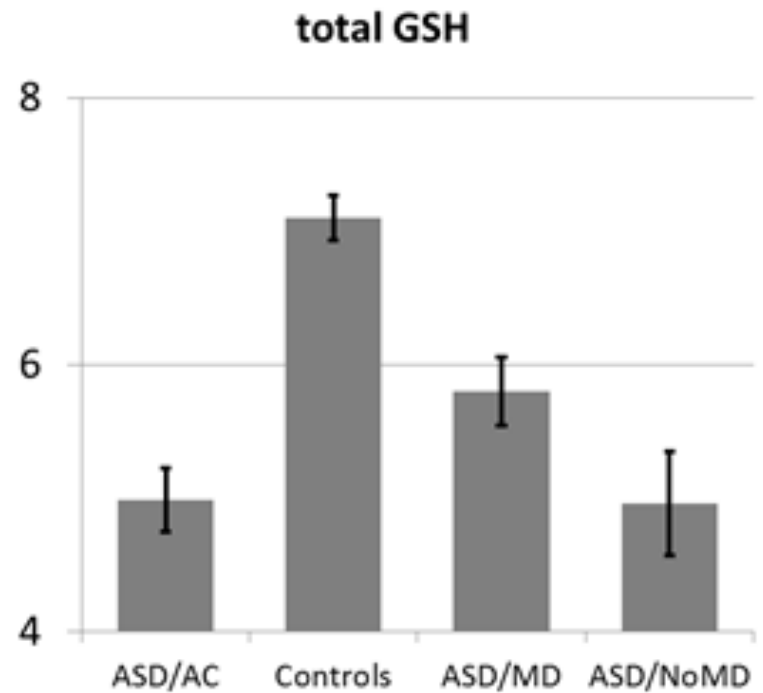




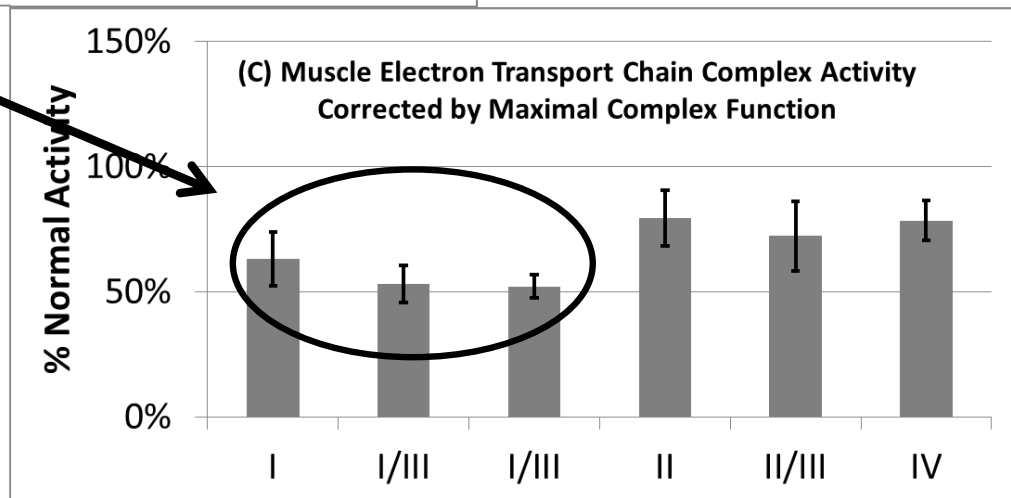
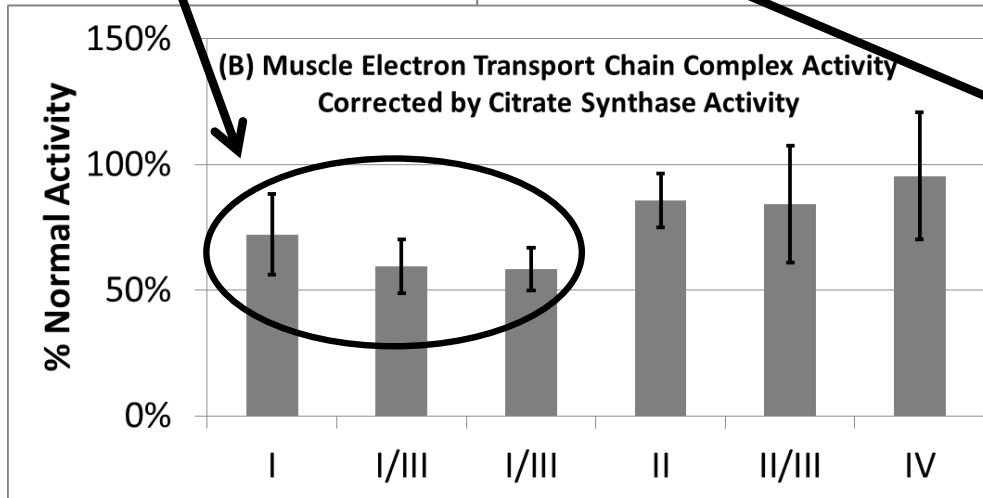
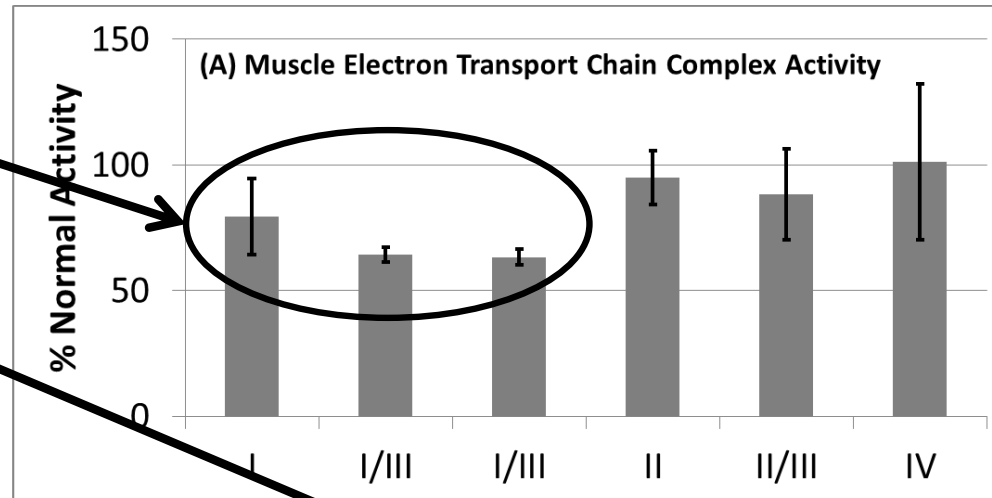
## Rodents

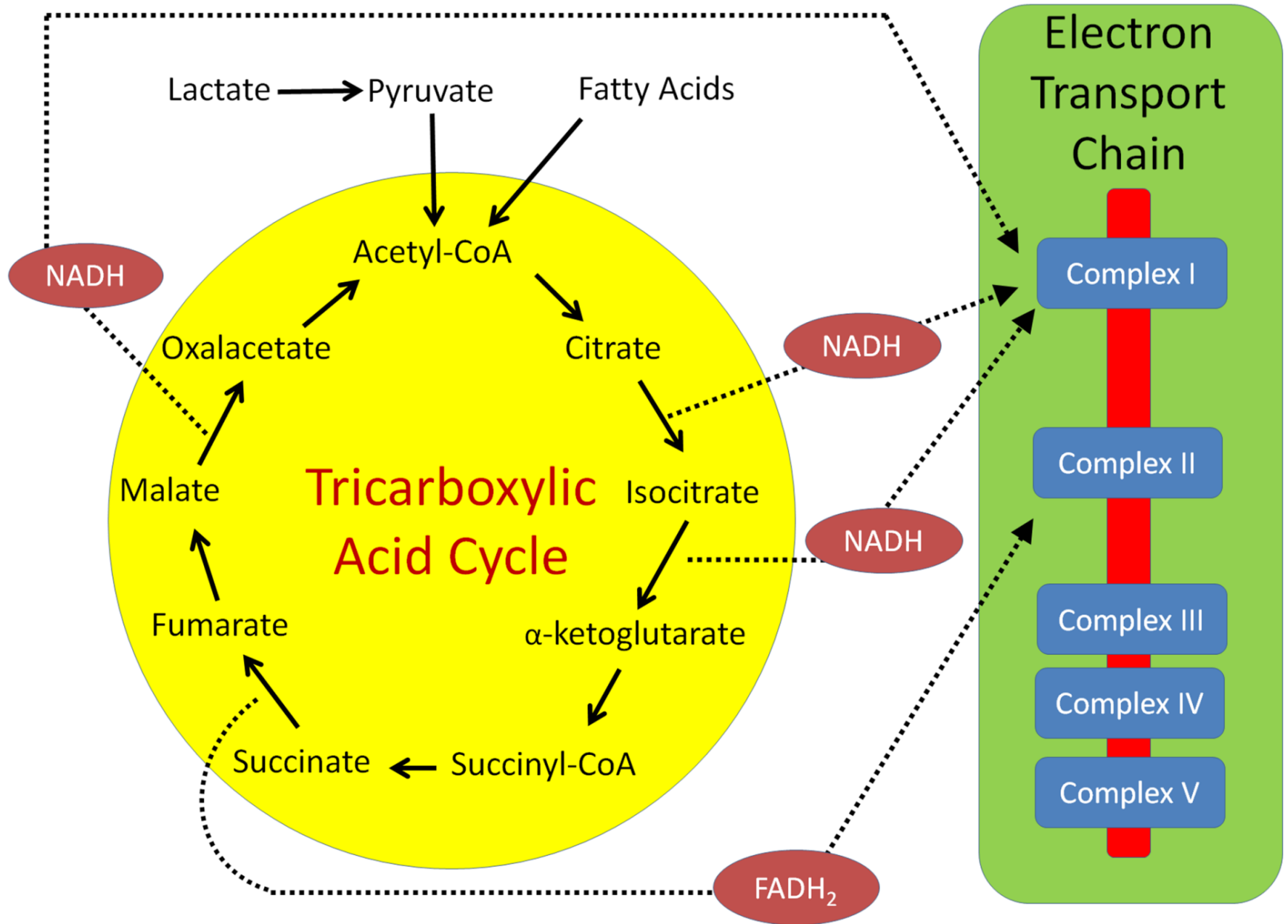


## ASD Children

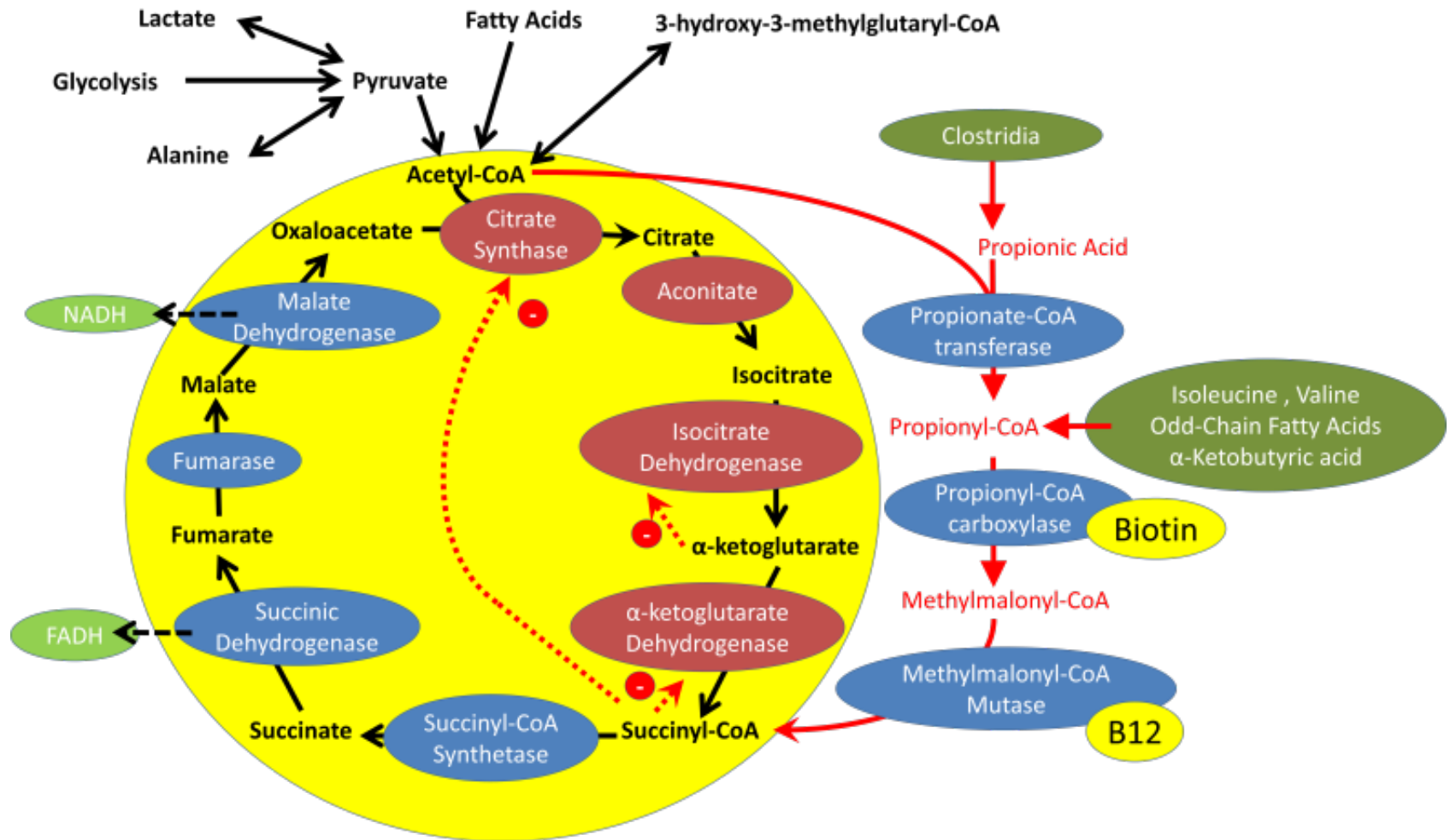


Decreased  
Complex I  
Activity





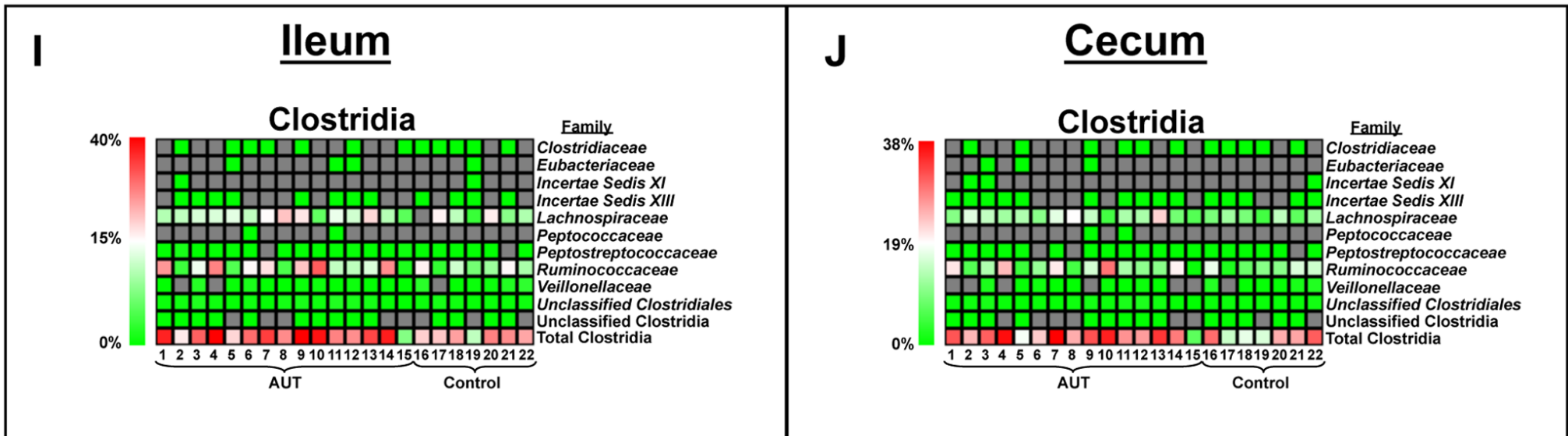




## Impaired Carbohydrate Digestion and Transport and Mucosal Dysbiosis in the Intestines of Children with Autism and Gastrointestinal Disturbances

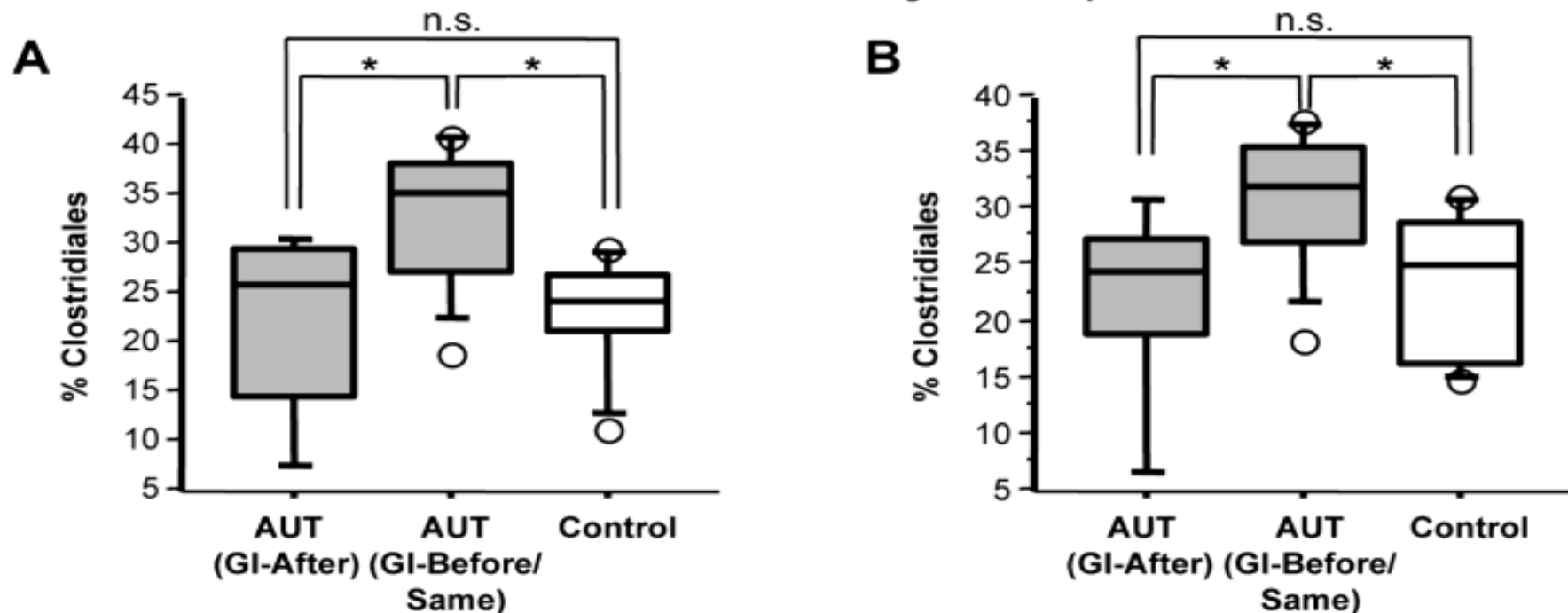
Brent L. Williams<sup>1</sup>, Mady Hornig<sup>1</sup>, Timothy Buie<sup>2</sup>, Margaret L. Bauman<sup>3</sup>, Myunghee Cho Paik<sup>4</sup>, Ivan Wick<sup>1</sup>, Ashlee Bennett<sup>1</sup>, Omar Jabado<sup>1</sup>, David L. Hirschberg<sup>1</sup>, W. Ian Lipkin<sup>1\*</sup>

<sup>1</sup> Center for Infection and Immunity, Columbia University, New York, New York, United States of America, <sup>2</sup> Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital, Boston, Massachusetts, United States of America, <sup>3</sup> Department of Neurology, Harvard Medical School and Departments of Neurology and Pediatrics and Learning and Developmental Disabilities Evaluation and Rehabilitation Services (LADDERS), Massachusetts General Hospital, Boston, Massachusetts, United States of America, <sup>4</sup> Department of Biostatistics, Columbia University, Mailman School of Public Health, New York, New York, United States of America



## Impaired Carbohydrate Digestion and Transport and Mucosal Dysbiosis in the Intestines of Children with Autism and Gastrointestinal Disturbances

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GI-After = GI Symptoms started after the onset of Autism symptoms

GI-Before/Same = GI Symptoms started before or at the same time as Autism symptoms





# Early Immune Activation

Model of Autism:

Animal Model and Probiotic Treatment



Cell

# Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,<sup>1,2,\*</sup> Sara W. McBride,<sup>1</sup> Sophia Hsien,<sup>1</sup> Gil Sharon,<sup>1</sup> Embriette R. Hyde,<sup>3</sup> Tyler McCue,<sup>3</sup> Julian A. Codelli,<sup>2</sup> Janet Chow,<sup>1</sup> Sarah E. Reisman,<sup>2</sup> Joseph F. Petrosino,<sup>3</sup> Paul H. Patterson,<sup>1,4,\*</sup> and Sarkis K. Mazmanian<sup>1,4,\*</sup>

<sup>1</sup>Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

<sup>2</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

<sup>3</sup>Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX 77030, USA

<sup>4</sup>These authors contributed equally to this work

\*Correspondence: [ehsiao@caltech.edu](mailto:ehsiao@caltech.edu) (E.Y.H.), [php@caltech.edu](mailto:php@caltech.edu) (P.H.P.), [sarkis@caltech.edu](mailto:sarkis@caltech.edu) (S.K.M.)

<http://dx.doi.org/10.1016/j.cell.2013.11.024>

- Antiphospholipid syndrome
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Psoriasis
- Type 1 diabetes
- Celiac disease
- Ulcerative colitis



- Preeclampsia
- Intrauterine growth restriction (IUGR)
- Maternal infection

## Maternal intrauterine inflammation

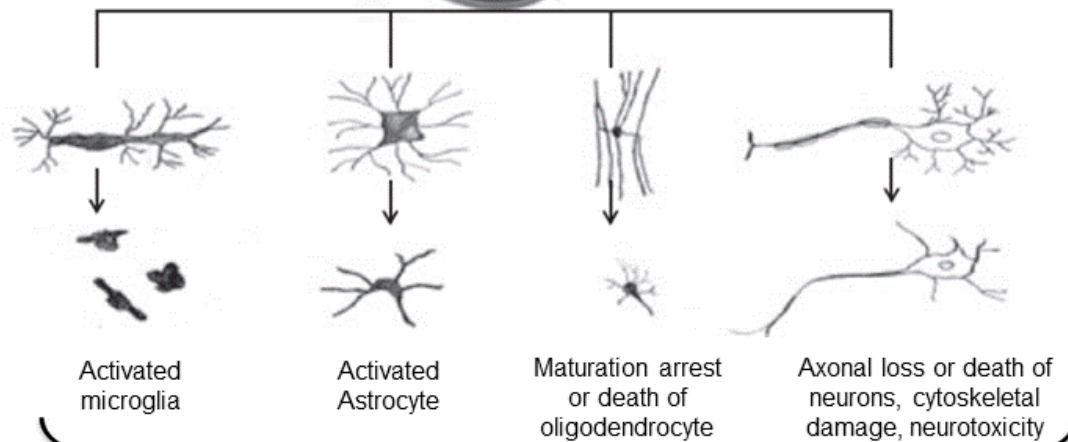
Increase in pro-inflammatory cytokines  
IL-6, IL-8, IFN- $\gamma$ , MCP-1, TNF- $\alpha$

Antibodies production:  
ANA (Ro, La), APA, anti-thyroid, anti-brain autoantibodies and anti paternal HLA alloantibodies

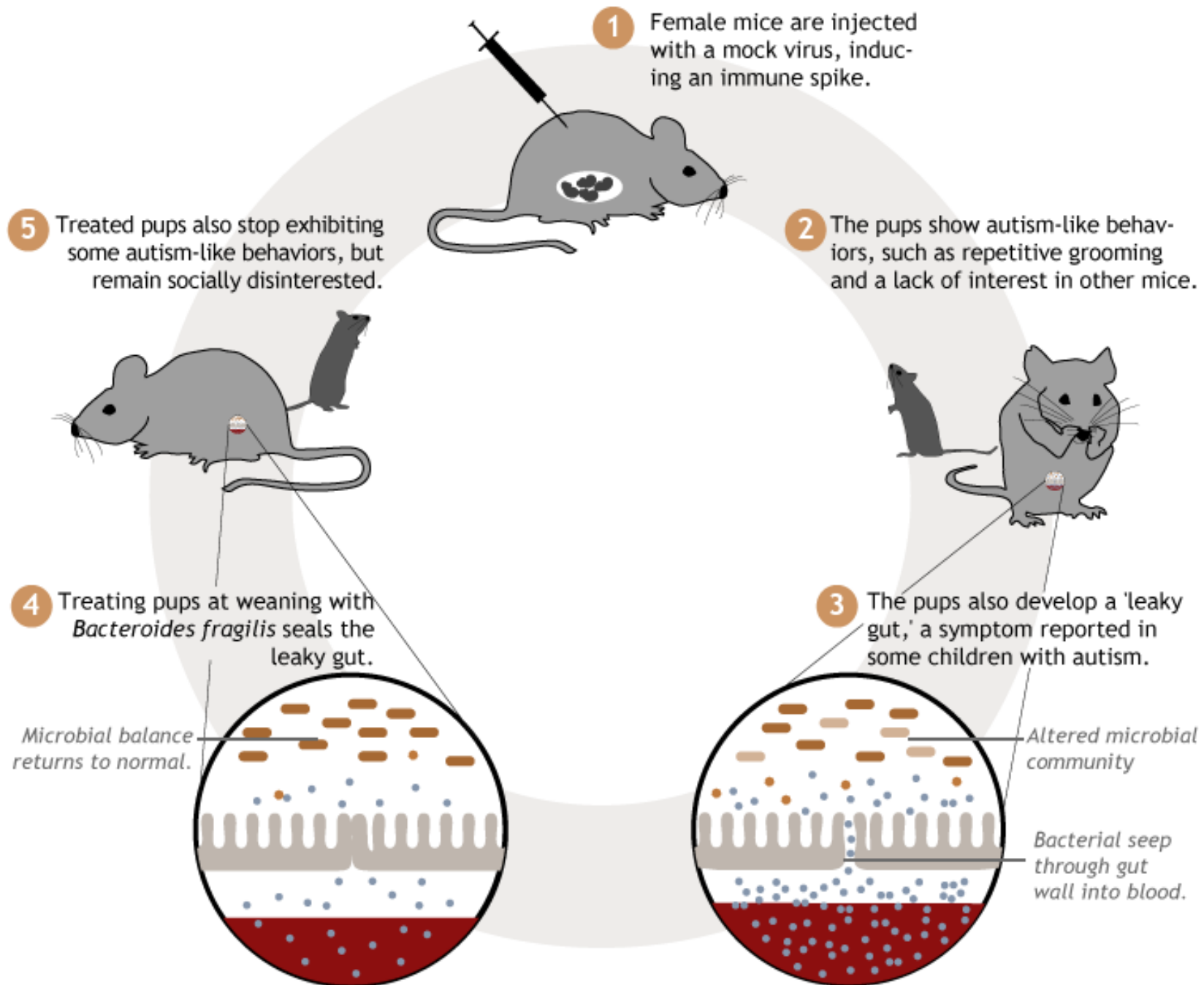
Preterm birth (LBWI)



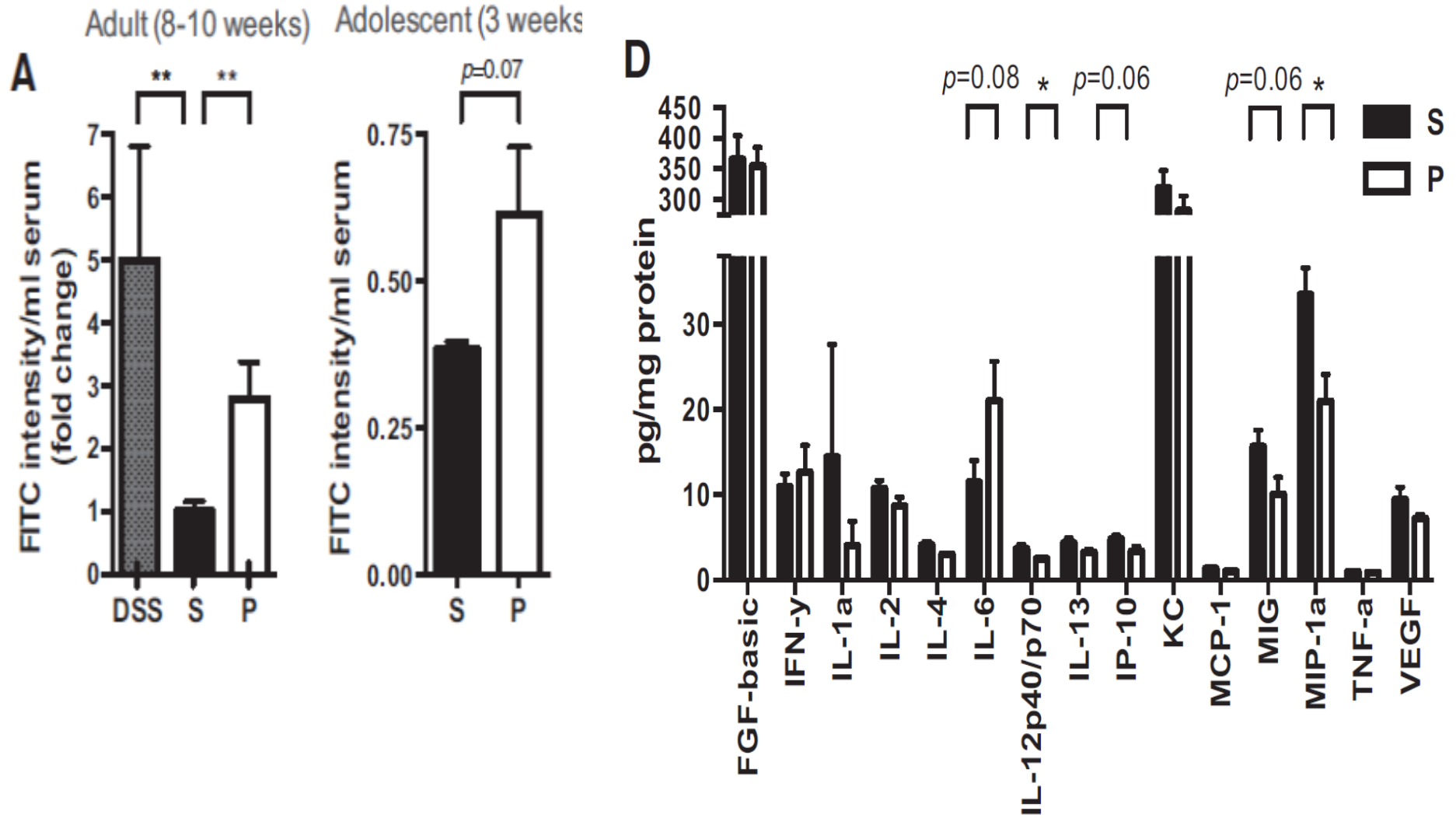
Fetal inflammatory response syndrome (increased proinflammatory cytokines in the brain)

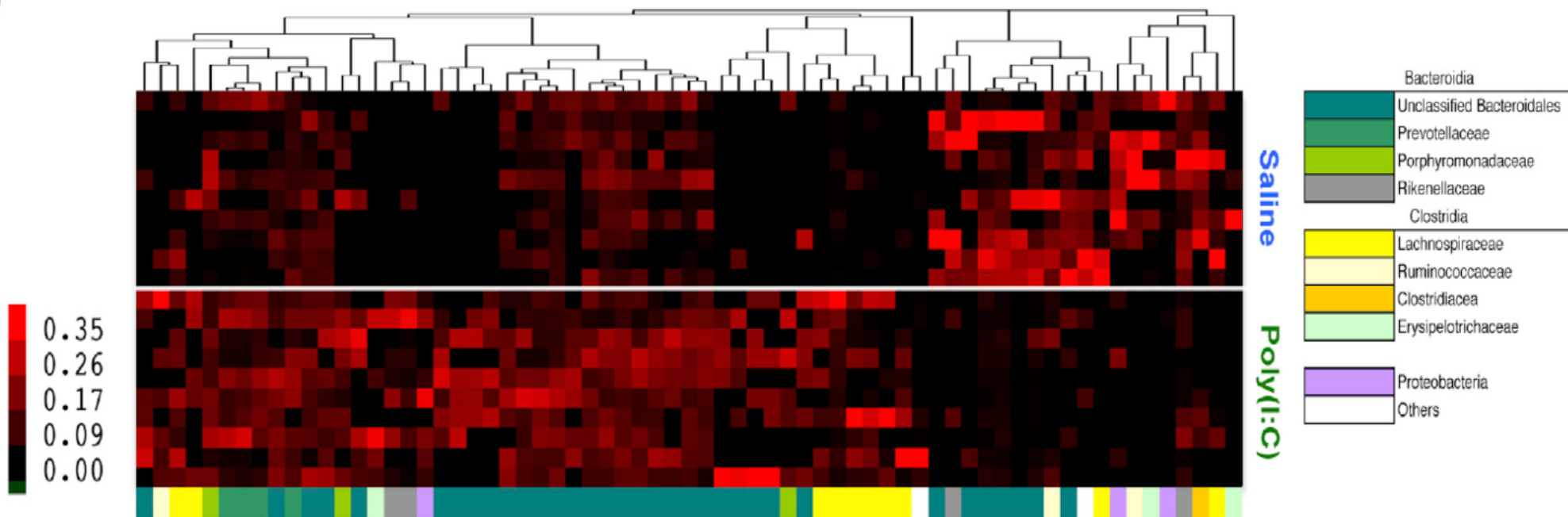


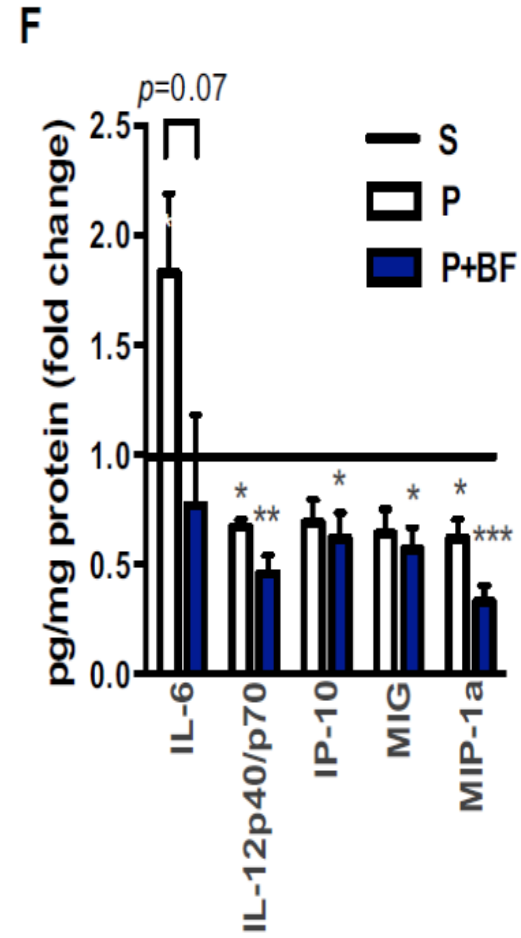
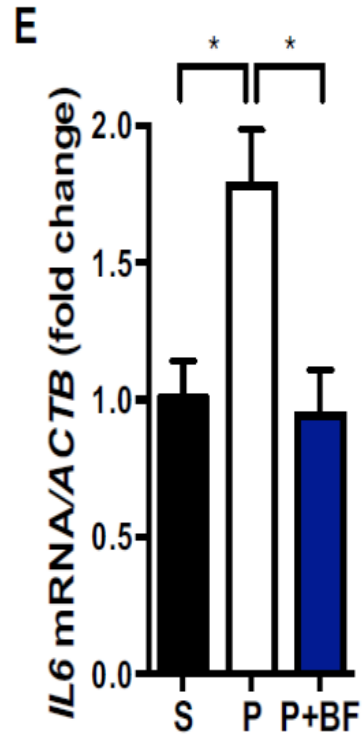
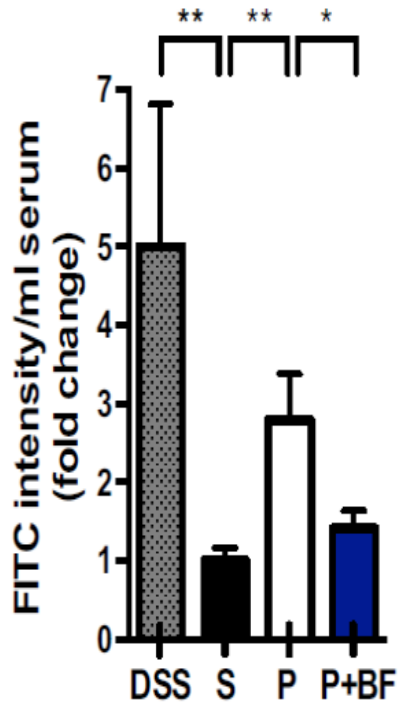
**Motor deficits, cognitive and behavioral impairment, psychiatric illness**







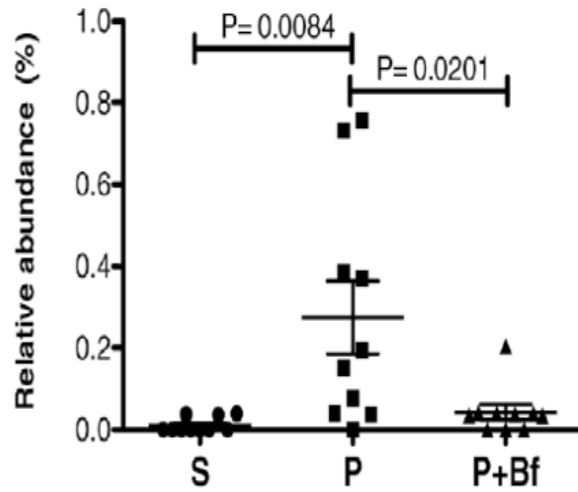




**B**

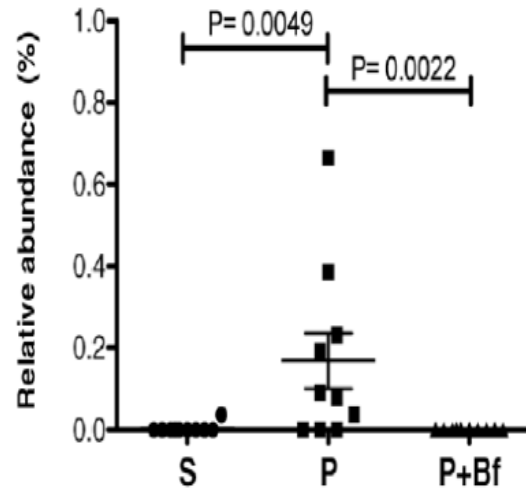
Lachnospiraceae

53

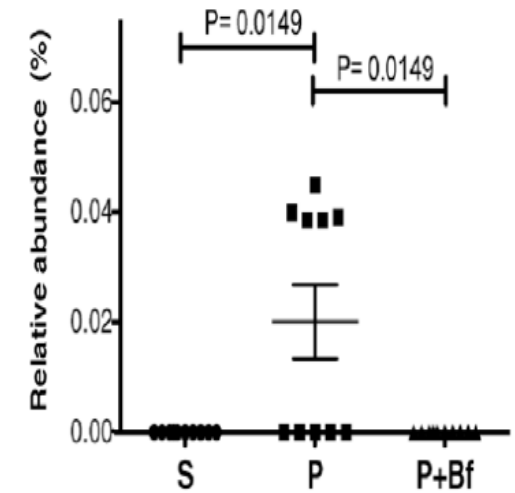


Bacteroidales

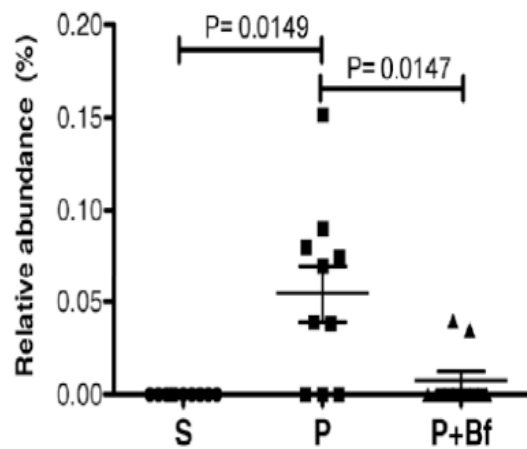
145



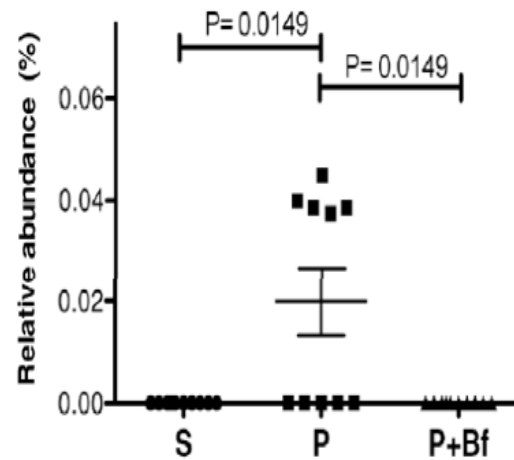
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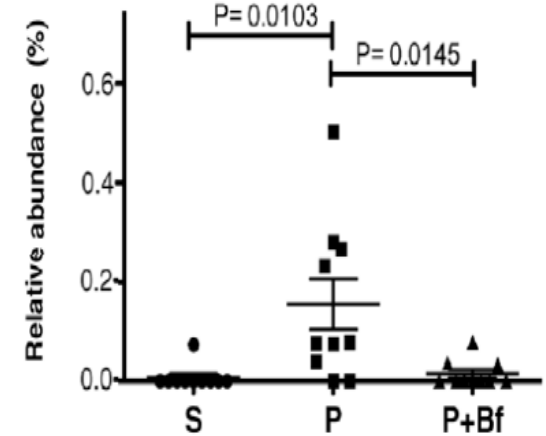
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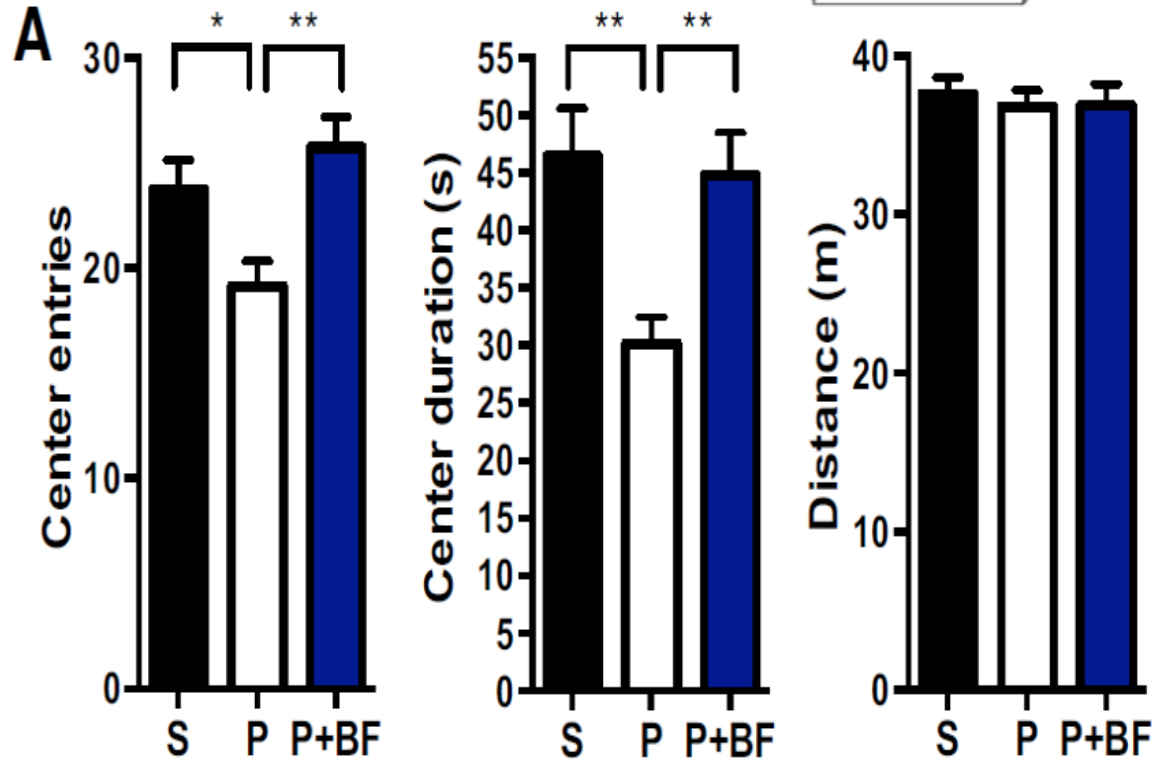
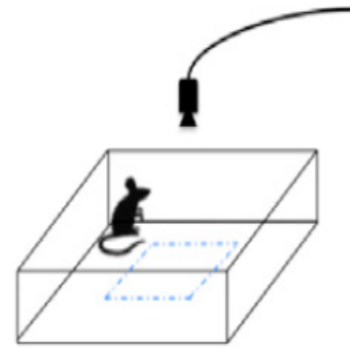


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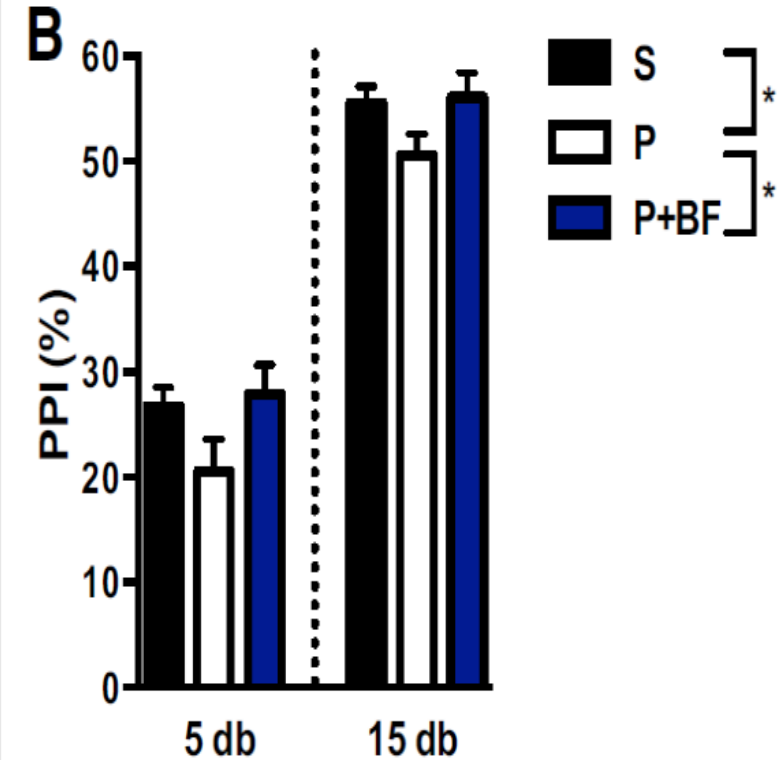
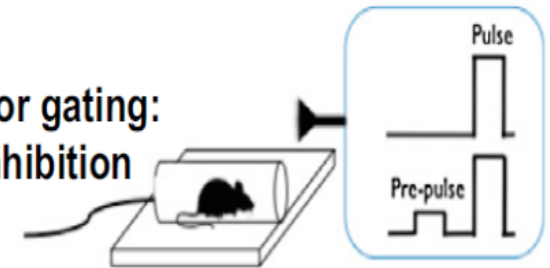




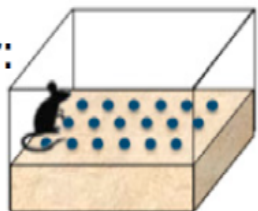
## Anxiety and locomotion: Open field exploration



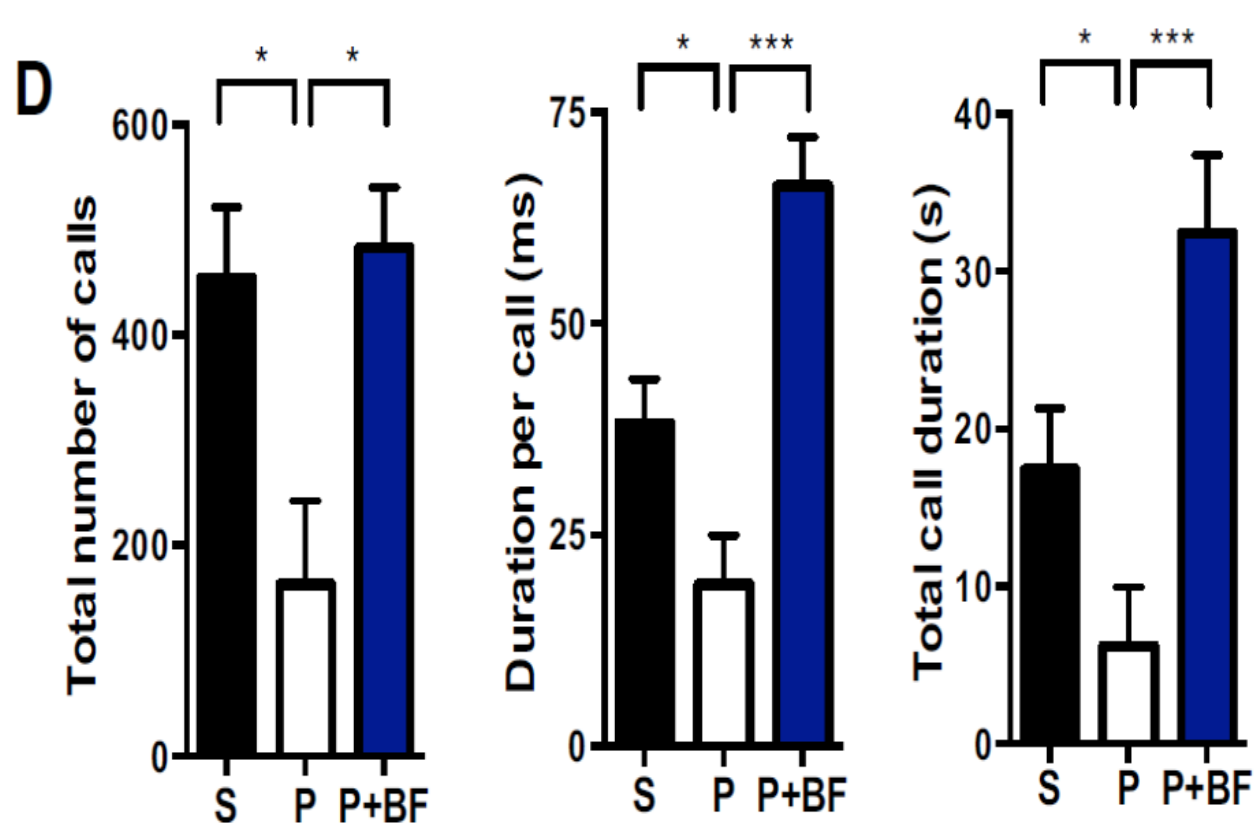
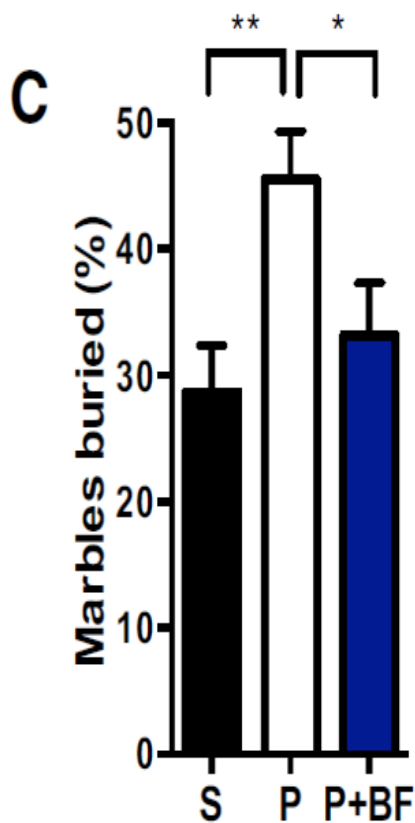
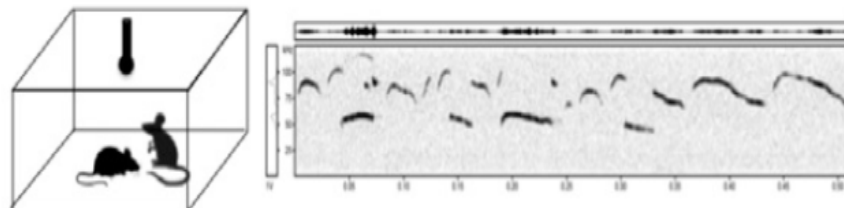
## Sensorimotor gating: Pre-pulse inhibition

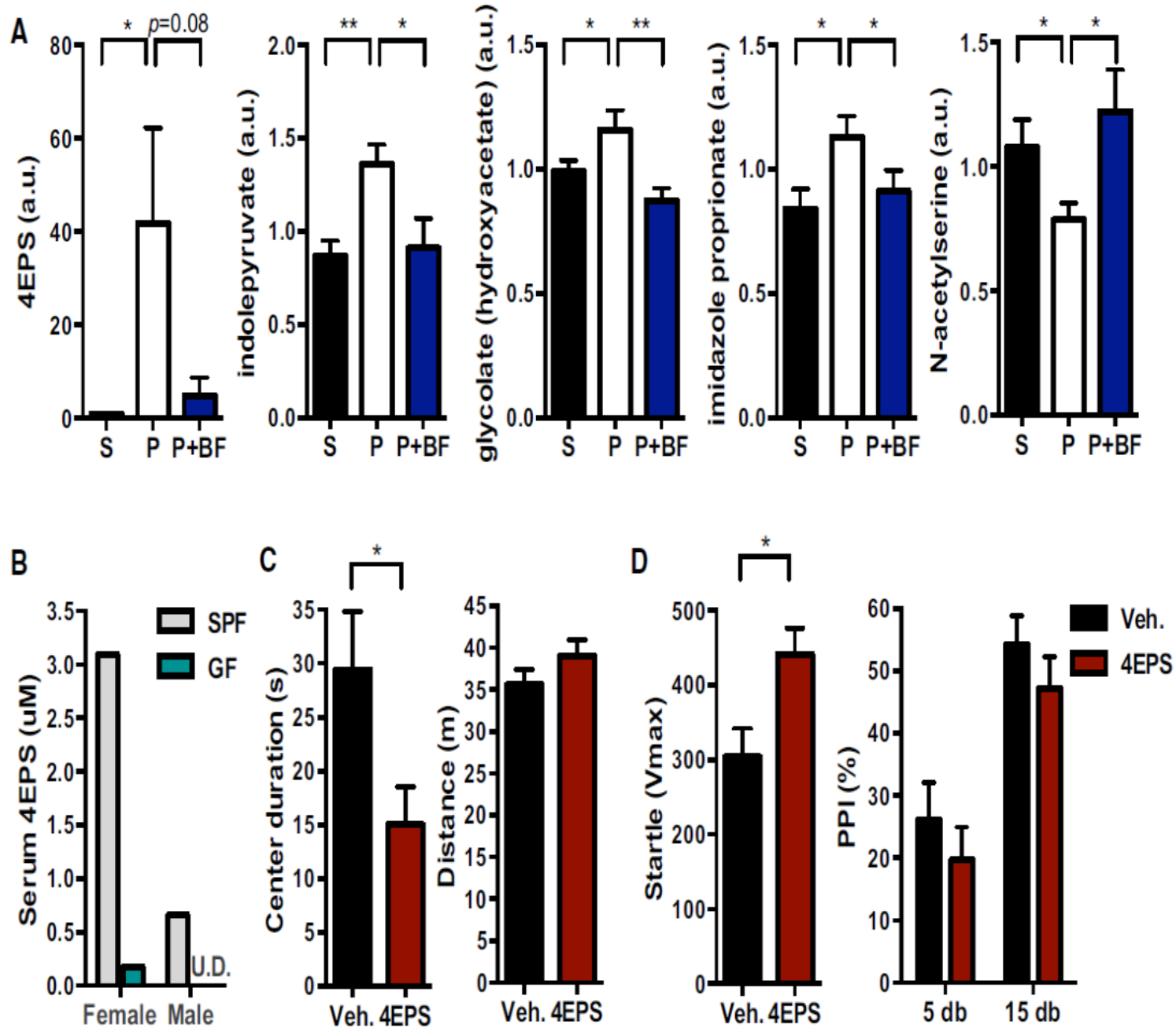


Stereotyped behavior:  
Marble burying



Communication:  
Ultrasonic vocalizations







## Microbiota Transfer Therapy (MTT)





Kang *et al. Microbiome* (2017) 5:10

DOI 10.1186/s40168-016-0225-7


## Microbiome

RESEARCH

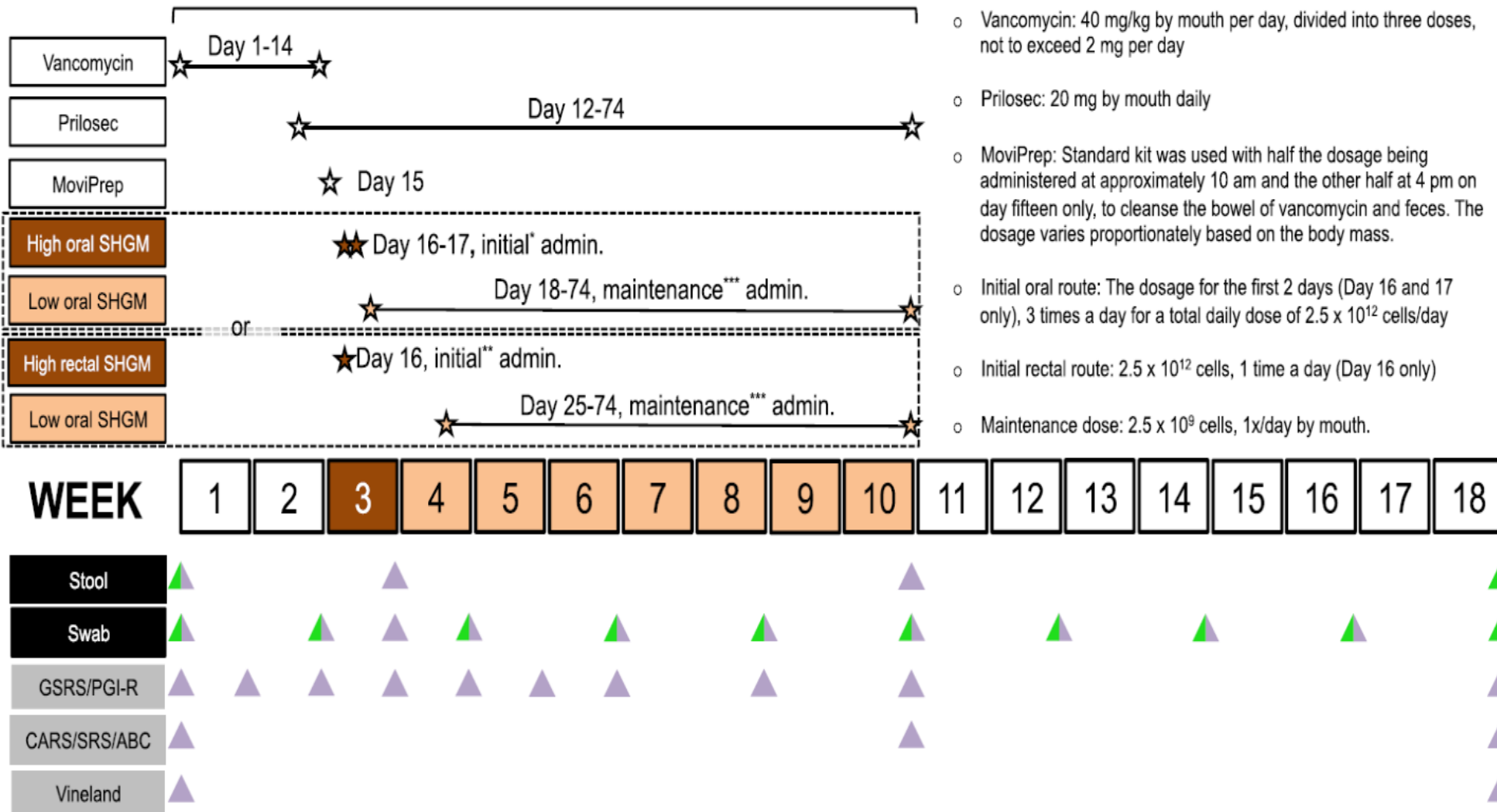
Open Access

# Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study

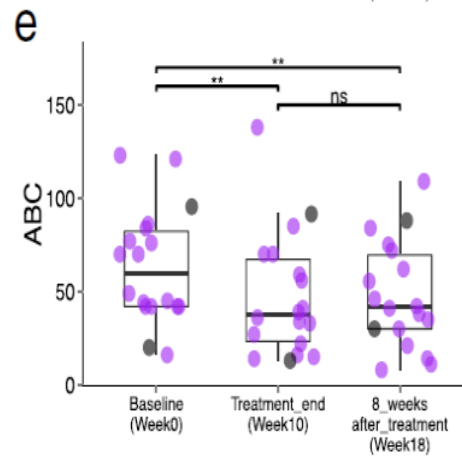
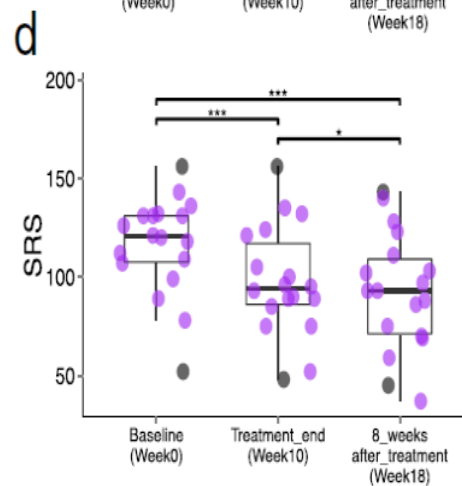
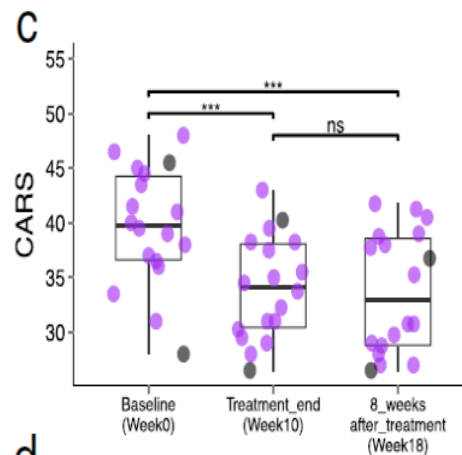
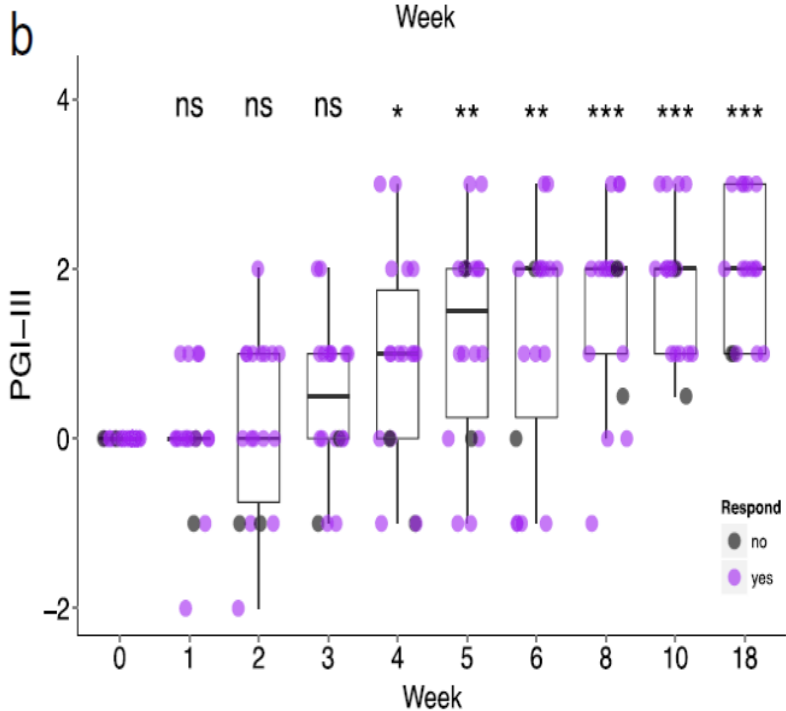
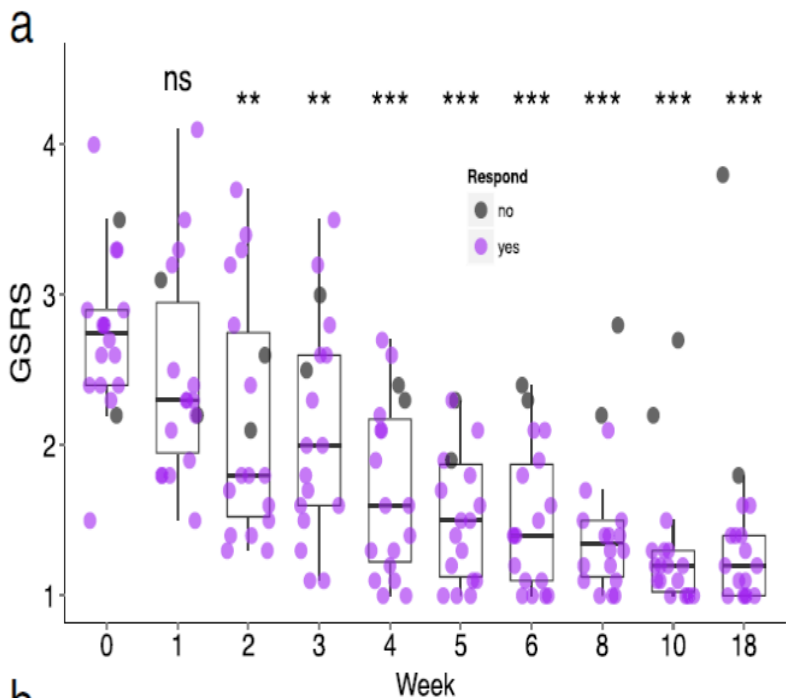


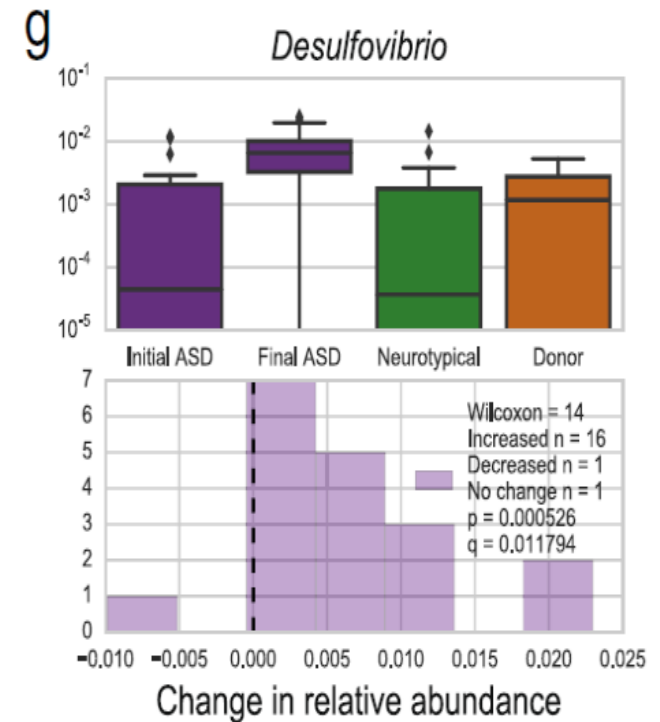
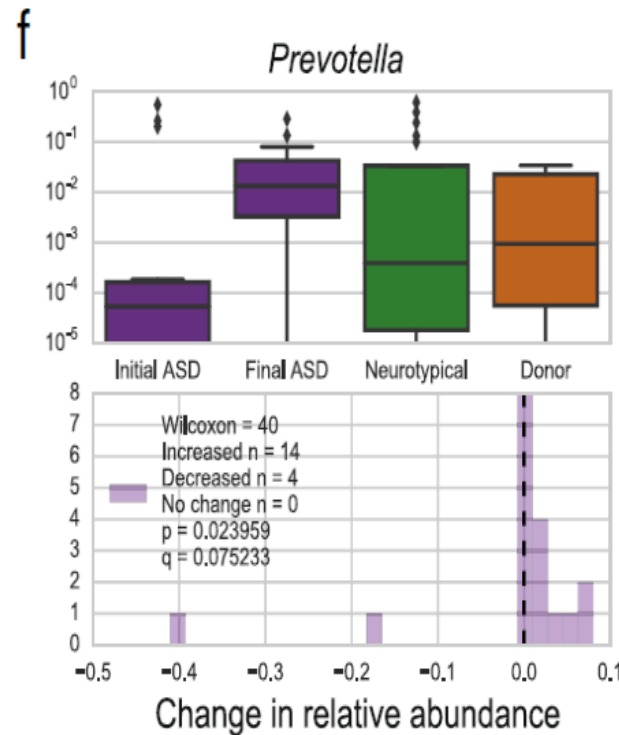
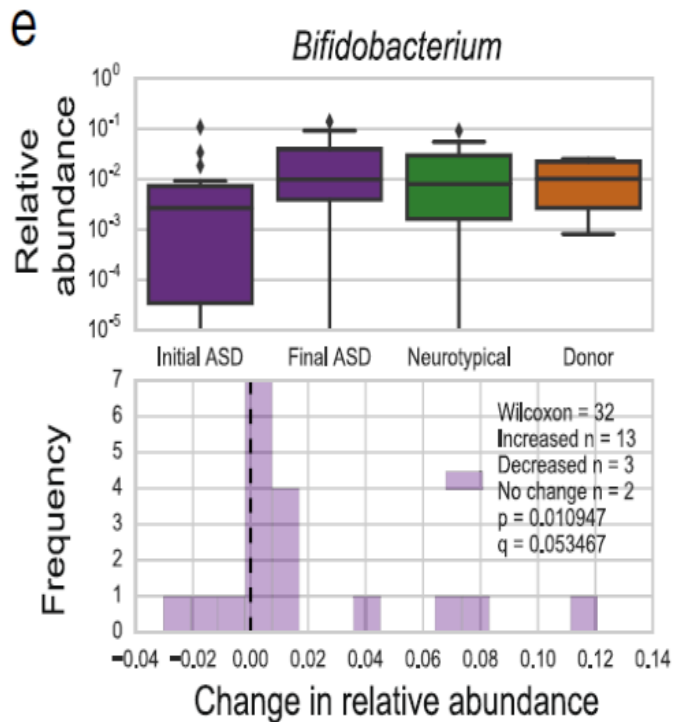
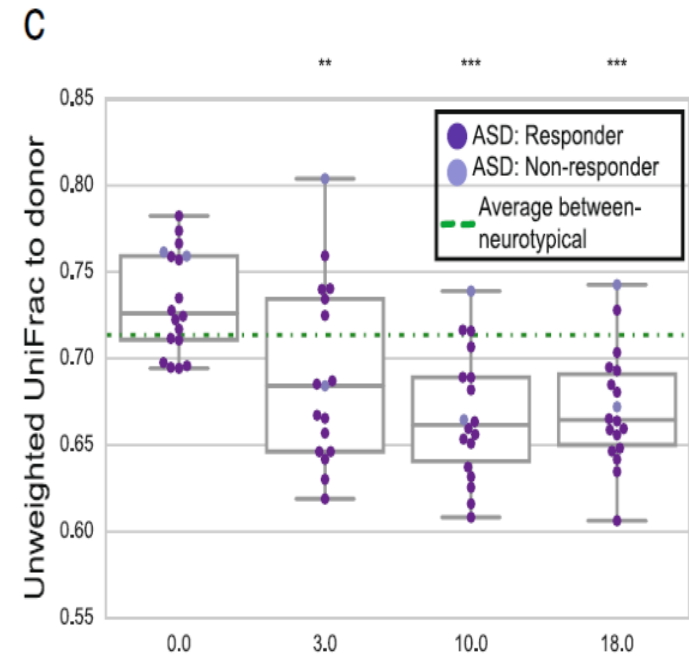
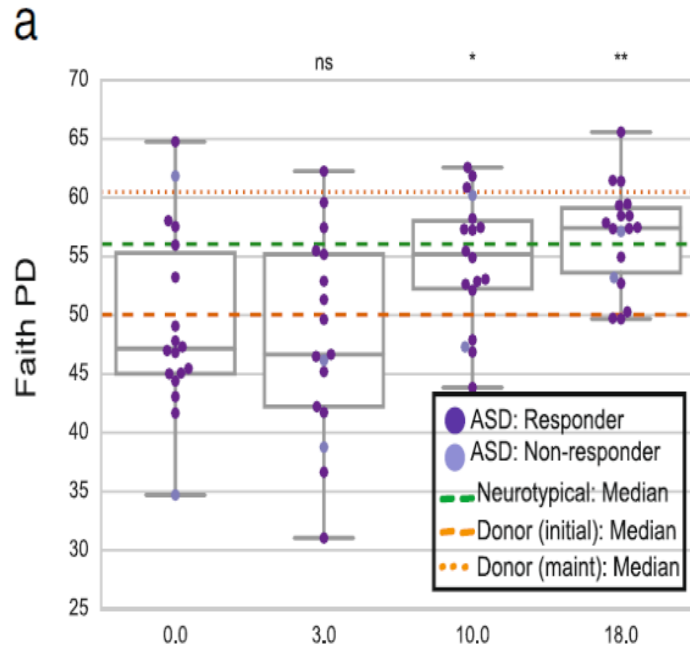
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## Microbiota Transfer Therapy (MTT)



**Fig. 1** Study design timeline. The trial consists of 10-week Microbiota Transfer Therapy (MTT) and 8-week follow-up observation period after treatment stopped. Schematic timeline represents a series of treatments that were performed during MTT (*top*) and frequencies of sample collection and GI/behavior assessments (*bottom*; neurotypical and ASD group colored in *green* and *purple*, respectively)







# Questions

