

MICROBIAL ENDOCRINOLOGY: HOW EVOLVED INTERSECTIONS OF MICROBIOLOGY AND NEUROBIOLOGY MATTER TO HEALTH AND SENSORY NUTRITION

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Structure of talk

- Introduction and recognition of bacteria as neuroendocrine organisms.
 - ▣ Consideration of microbiota from neuroendocrine vantage point allows introduction of new approaches to understanding role in sensory nutrition.
 - How it started and taking an evolutionary vantage point.
- What does this mean for understanding the complex interactions between host, diet and microbiota that may have a role to play in sensory nutrition.
 - ▣ Talk will not be comprehensive review but instead introduce one of the ways forward.
- What is *really* happening – hype vs. reality.
 - ▣ Potential, problems and paths forward – one (of many) will be discussed.
- Concept of neuroendocrine-bacterial interactions has been termed ***Microbial Endocrinology***.

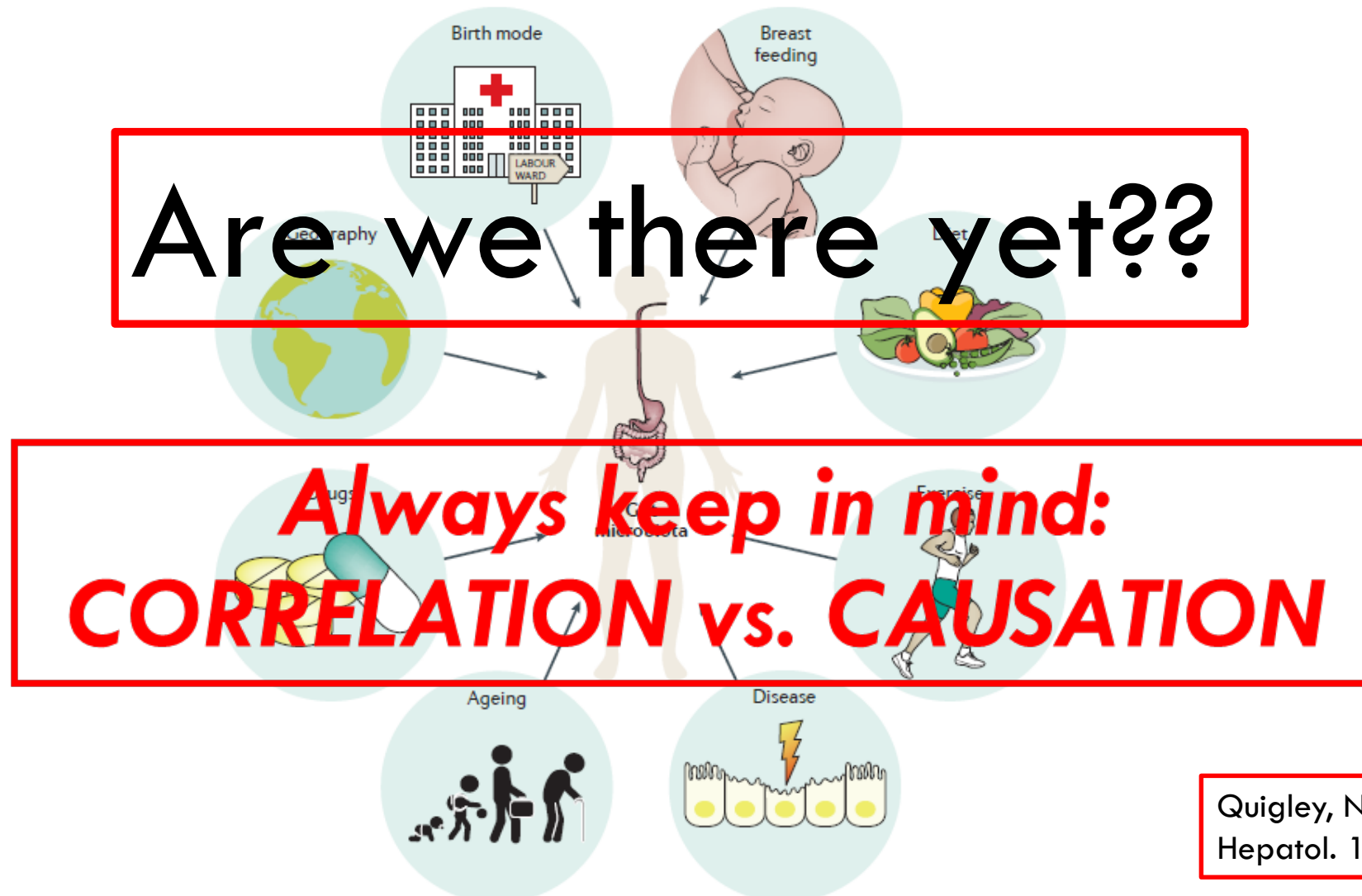
To keep in mind

- Evolutionary-based approach linking the components of nutrition with the microbiome and its interface with the host.
 - ▣ Ever before anything gets “into” you or an animal, it first comes in contact with the microbiota.
- Use of neurochemistry as a “**common evolutionary language**” in which all elements, host, microbiota and nutritive, interact.
- Neurochemicals in food as regards the microbiota, but they can also directly influence the host immune response.
- It is fully recognized that microbial endocrinology is one of the **possible** mechanisms and that a vast array of other possibilities exist and need to be explored.

Current state of microbiome and sensory nutrition

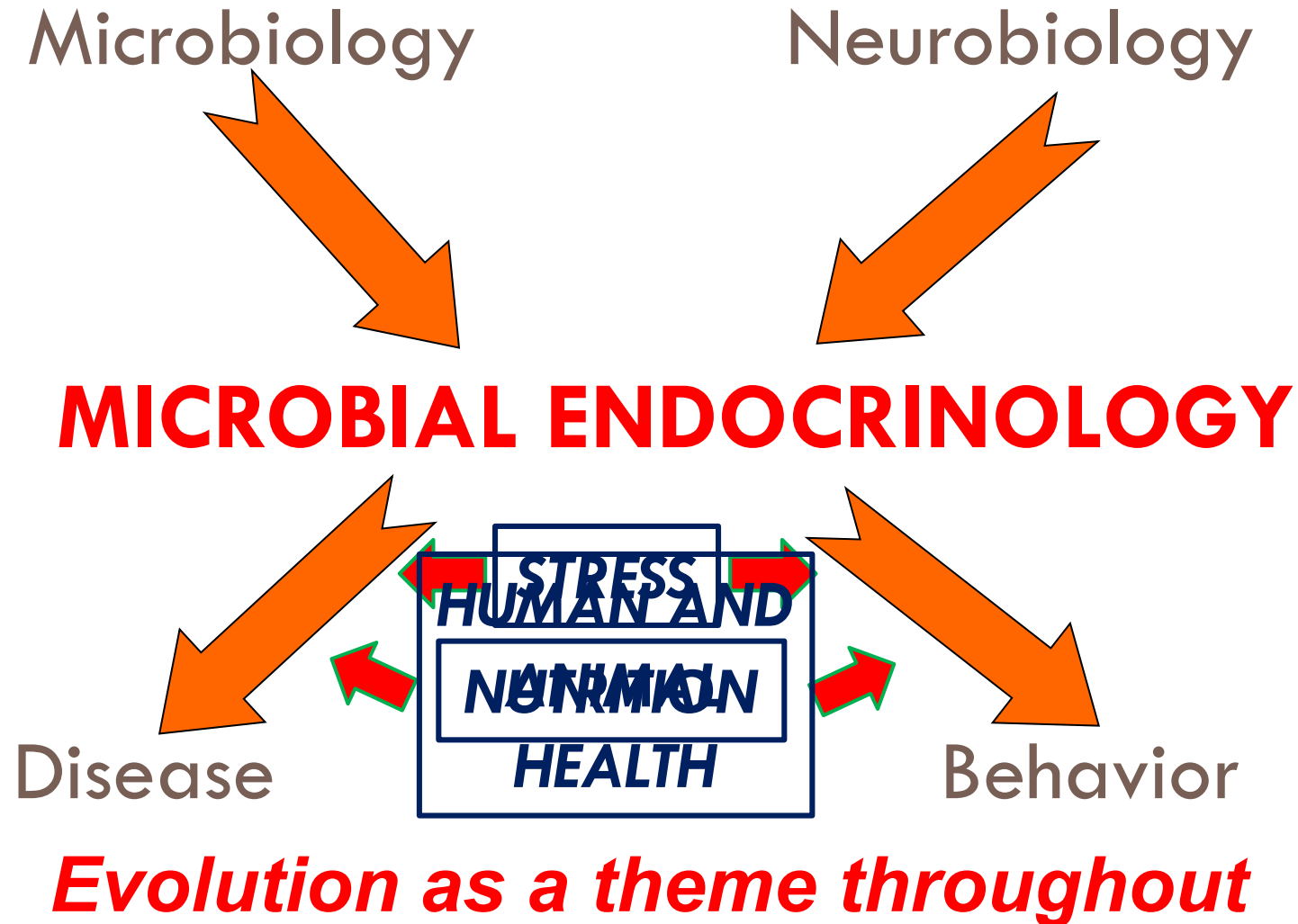
- Studies date back many decades which have shown that microbes can produce chemicals that influence behavior in insects and mammals.
- Demonstration of mechanistic pathways by microbes may produce odorants that can be used by hosts as olfactory signals thereby influencing sensory nutrition and behavior.
- Excellent reviews (among the many) can be found:
 - ▣ Bienenstock *et al.* Disruptive physiology: olfaction and the microbiome-gut-brain axis. *Biological Reviews* 93: 390-403, 2018.
 - ▣ van de Wouw *et al.* Microbiome-gut-brain axis: Modulator of host metabolism and appetite. *Journal of Nutrition* 147:727-45, 2017.
- Omics-based strategies cannot get you complete picture.
 - ▣ Need for “old school” microbiology and nutritionally relevant medium approach.

Extent of microbiome effects on host



Quigley, Nat. Rev. Gastroenterol.
Hepatol. 14:315-320, 2017

Microbial endocrinology defined



What led to development of microbial endocrinology?



IS THIS THE COMPLETE STORY?

Testing theory of stress-immune interactions

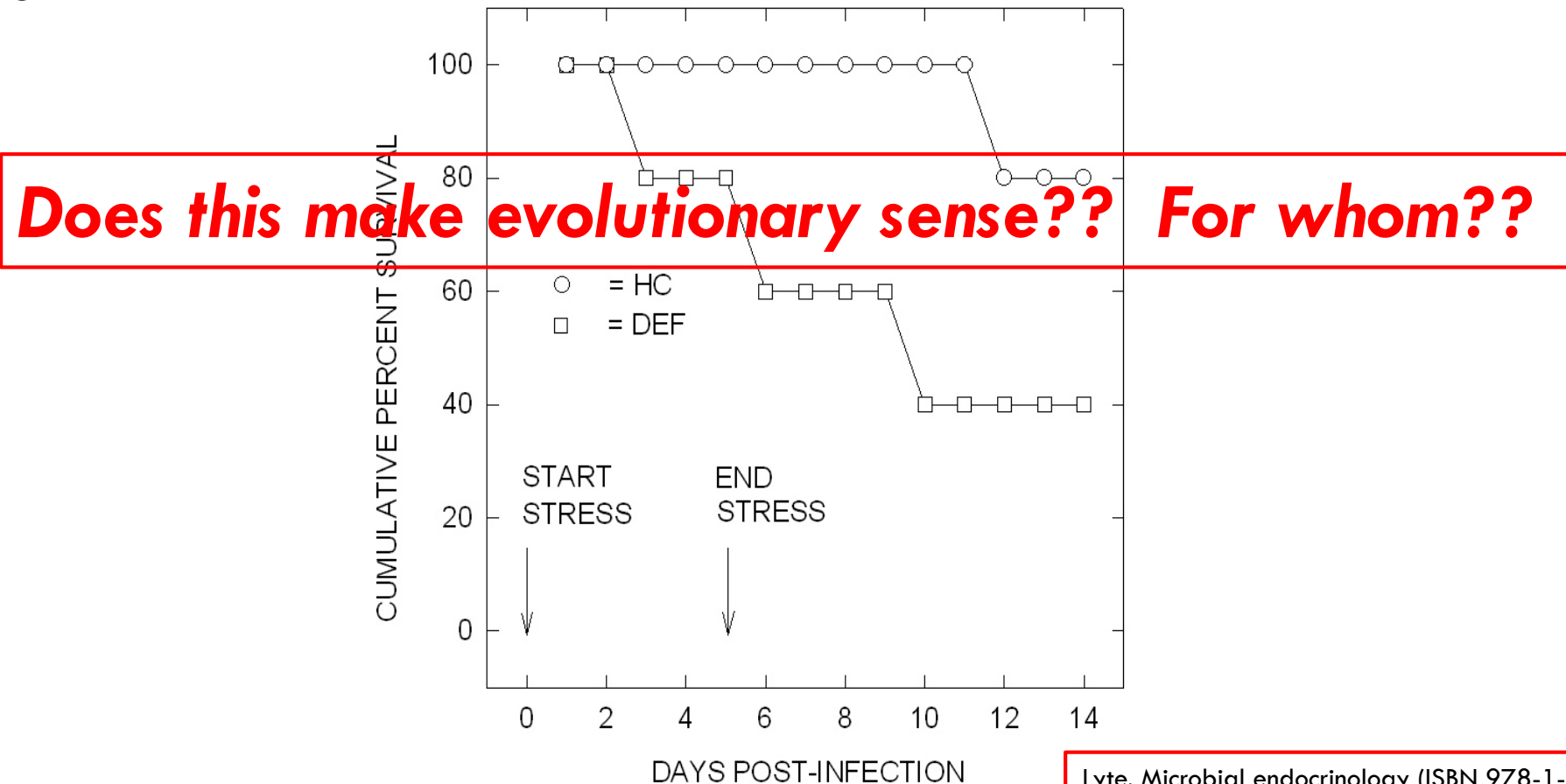


macrophage cytotoxicity increased >500%

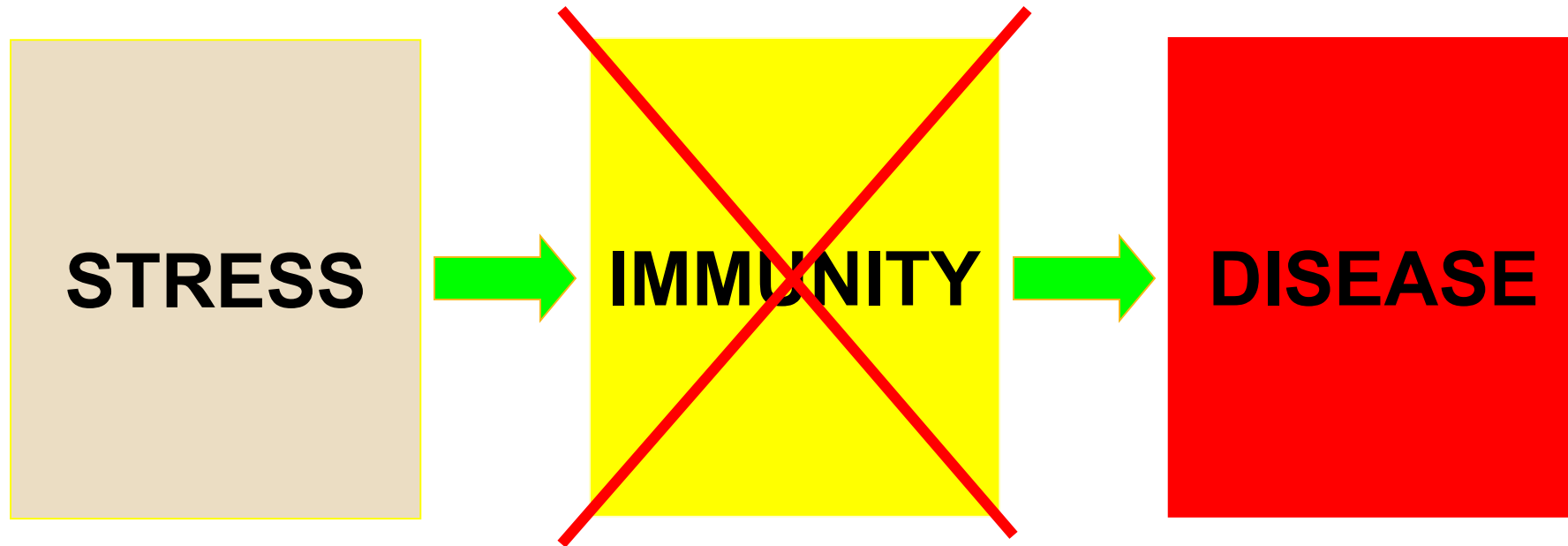


Is stress-induced increase in immune response protective?

- Social conflict stress and then gave orally the common food pathogen *Y. enterocolitica*.



Stress-immune interactions - reconsidered



***Whose survival are we talking about?
Are microorganisms neurochemical
responsive organisms?***

Presence of neurochemicals in food

□ Bananas

- ▣ 700 µg/g dopamine and 70 µg/g norepinephrine
- ▣ Division between peel and pulp
 - Waalkes *et al.*, Science 127:648, 1958.

□ Tribal pulses

- ▣ 8 g of L-Dopa per 100 g of flour
- ▣ Resistant to destruction by autoclaving and boiling

□ Other common foodstuffs

- ▣ Tomatoes - dopamine, tyramine
 - Use of psychoactive drugs and MAOI restrictive diets
- ▣ Cheese - tyramine

Presence of neurochemicals in the microbial world

□ Bacteria

- Insulin-like material - present in all strains examined
- GABA – Probiotics as well as clinical bacterial pathogens
- Somatostatin - *Bacillus subtilis*
- Catecholamines - *E. coli*
- Specific receptors have been demonstrated - 100% homology of *E. coli* EnvY gene for high affinity opioid binding site.

Do neurochemicals affect bacteria?

□ Protozoa

- Catecholamines - *Crithidia fasciculata*, *Paramecium*
- Serotonin - *Tetrahymena pyriformis*

□ Fungi

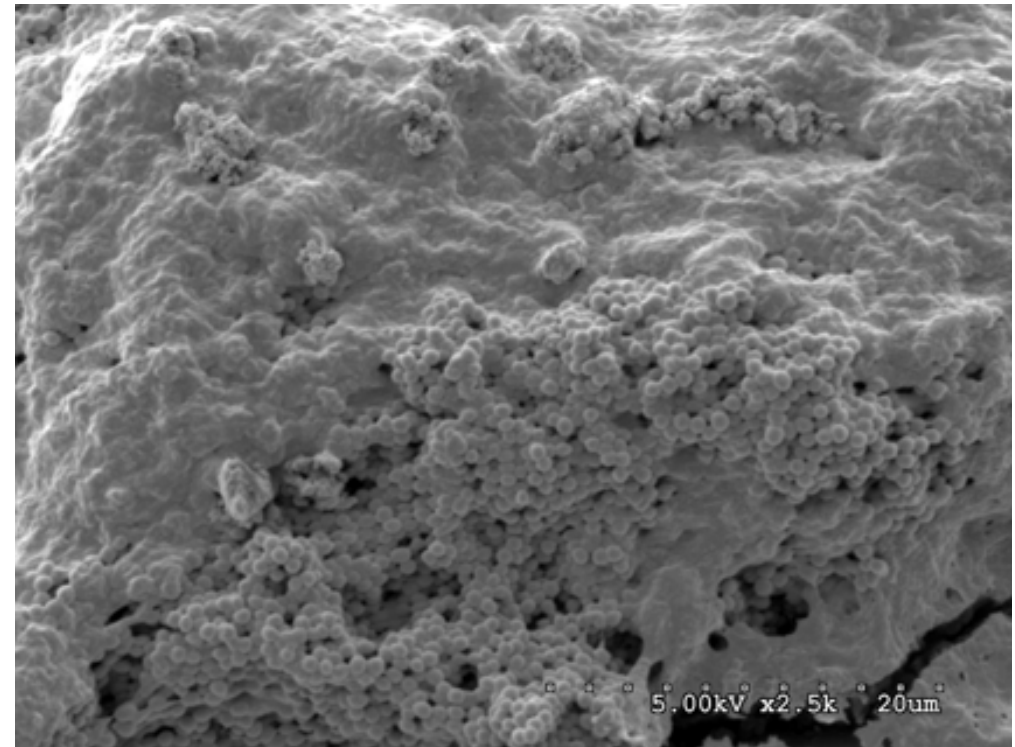
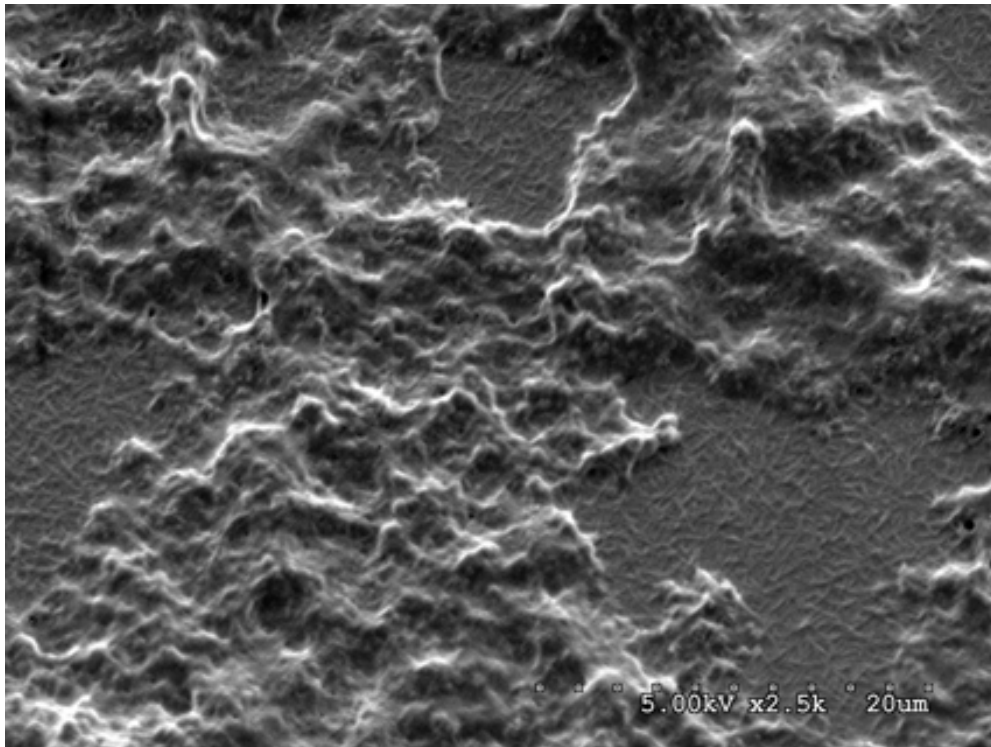
- Sex pheromone - Truffles (Androstenol)

Real-world relevance of microbial endocrinology

Mechanisms of disease

Lancet 361:130-5, 2003

Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes



Common theoretical thread

- There is an evolutionary relationship between microorganisms and host.
- Evolution of cell-cell signaling in animals may be due to late horizontal gene transfer from bacteria.
 - ▣ Iyer *et al.*, Trends in Genetics 20:292, 2004.
- Microorganisms, such as those in the gut (really everywhere), do not simply rely on traditional nutritive (energy) sources for their survival and behavior.
- Concept of direct neuroendocrine-bacterial interactions means bacteria interactive player in health and nutrition.

Where we have come from...

- Emergence of human microbiome and what it means for behavior in a microbiota-gut-brain axis:
 - ▣ From 1914: *"The control of man's diet is readily accomplished, but mastery over his intestinal bacterial flora is not... They are the cases that present...malaise, total lack of ambition so that every effort in life is a burden, mental depression often bordering upon melancholia...A battle royal must be fought and when this first great struggle ends in victory for the Bacillus bulgaricus it must be kept on the field of battle forever at guard..."*
 - Stow, Medical Record Journal of Medicine and Surgery, 1914

Gut – *Where neurochemicals and bacteria meet*

- Production and metabolism of norepinephrine and dopamine within mesenteric organs over 50% total body.
 - ▣ Åneman *et al.*, J. Clin. Invest. 97:1640, 1996; Eisenhofer *et al.*, Am. J. Physiol. 268:G641, 1995.
- Within lumen of GI tract physiologically relevant levels of hormones:
 - ▣ Serotonin release from enterochromaffin cells.
 - ▣ Norepinephrine and dopamine.
- Dietary sources:
 - ▣ Foods are a rich source of neuroendocrine hormones

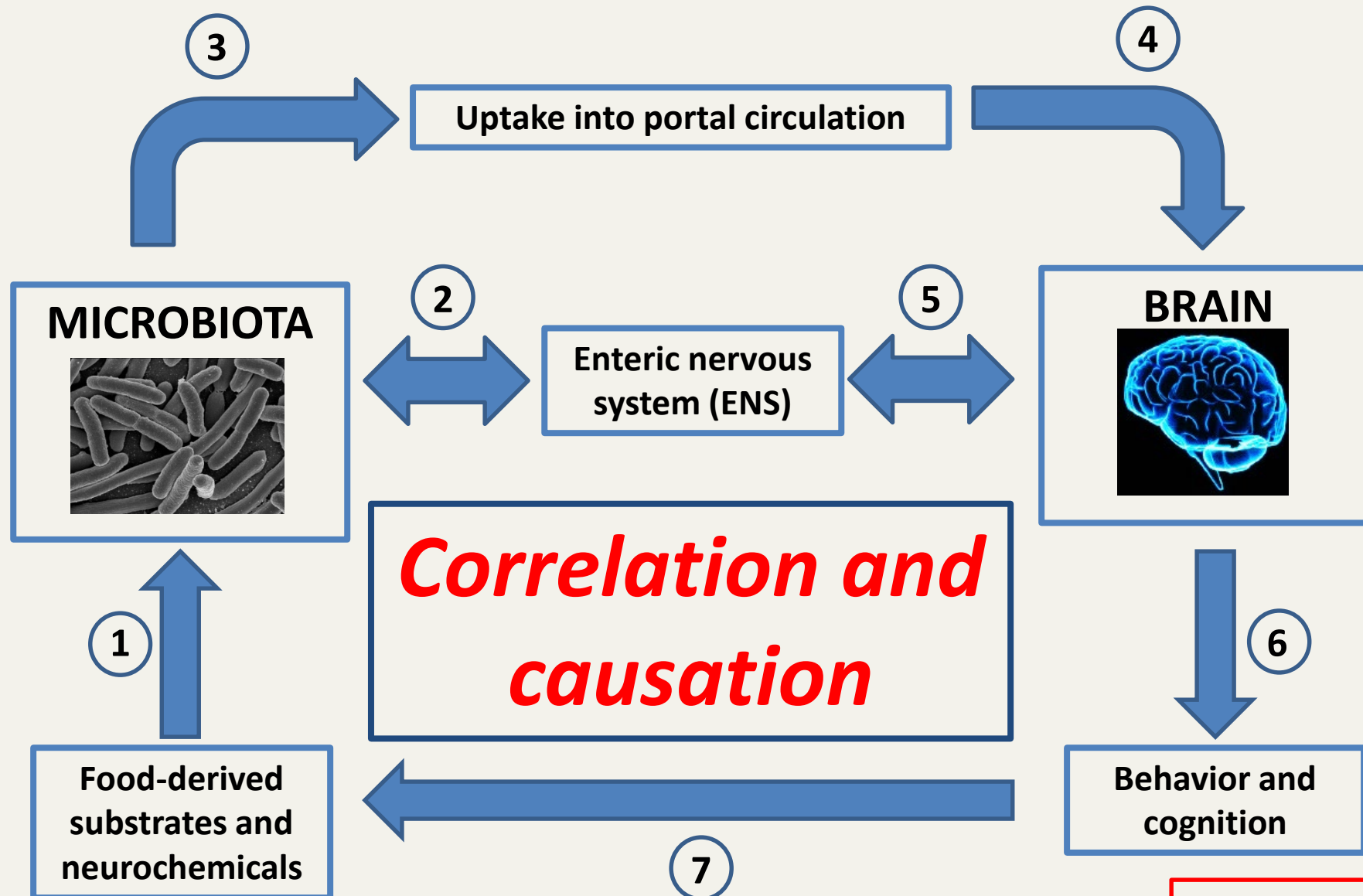
ENS innervation of gut and proximity to microbiota

Vagal villus
afferents

Question is
where does
information
flow and
*possible bi-
directionality*



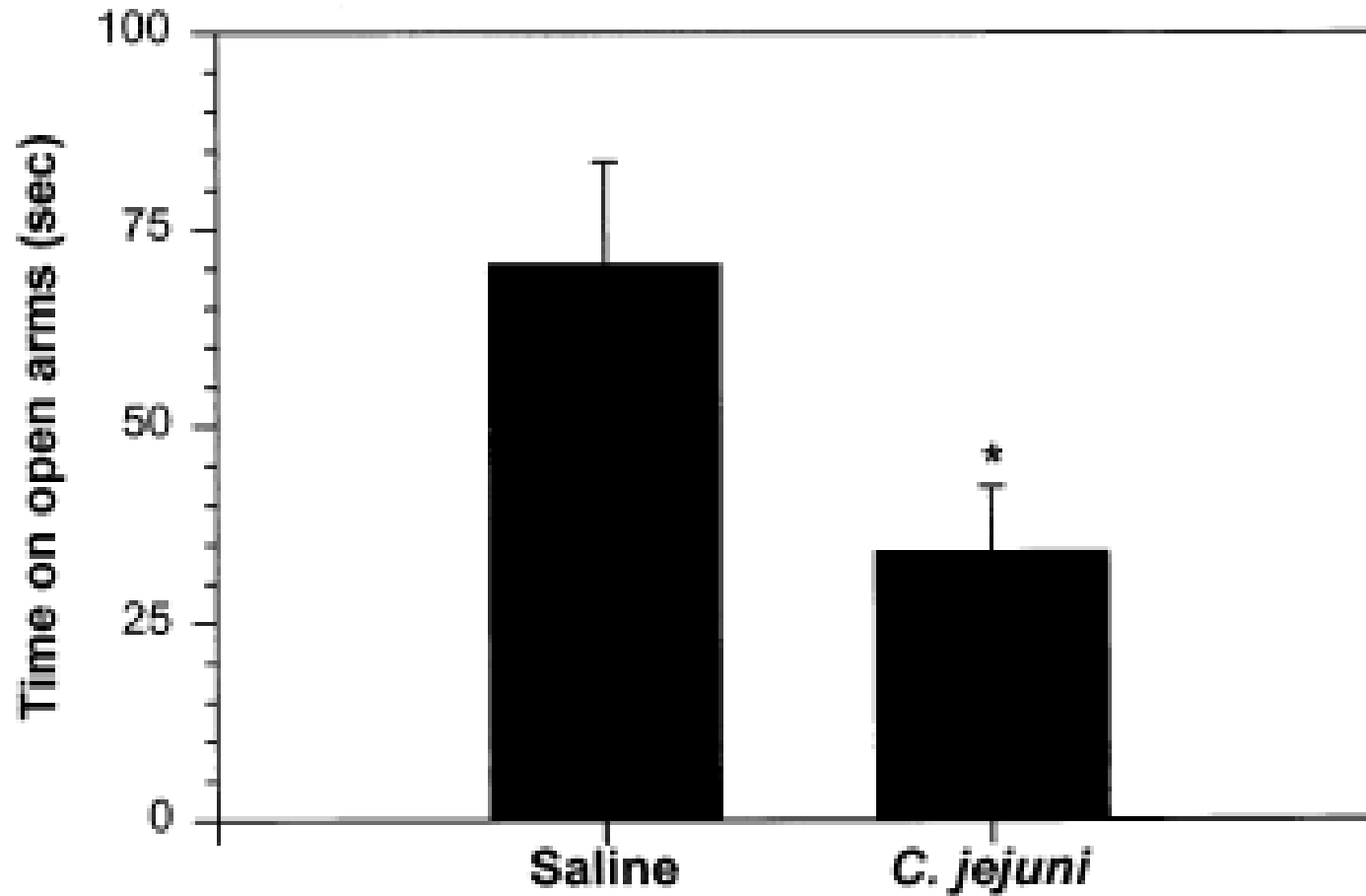
Microbiota-gut-brain axis



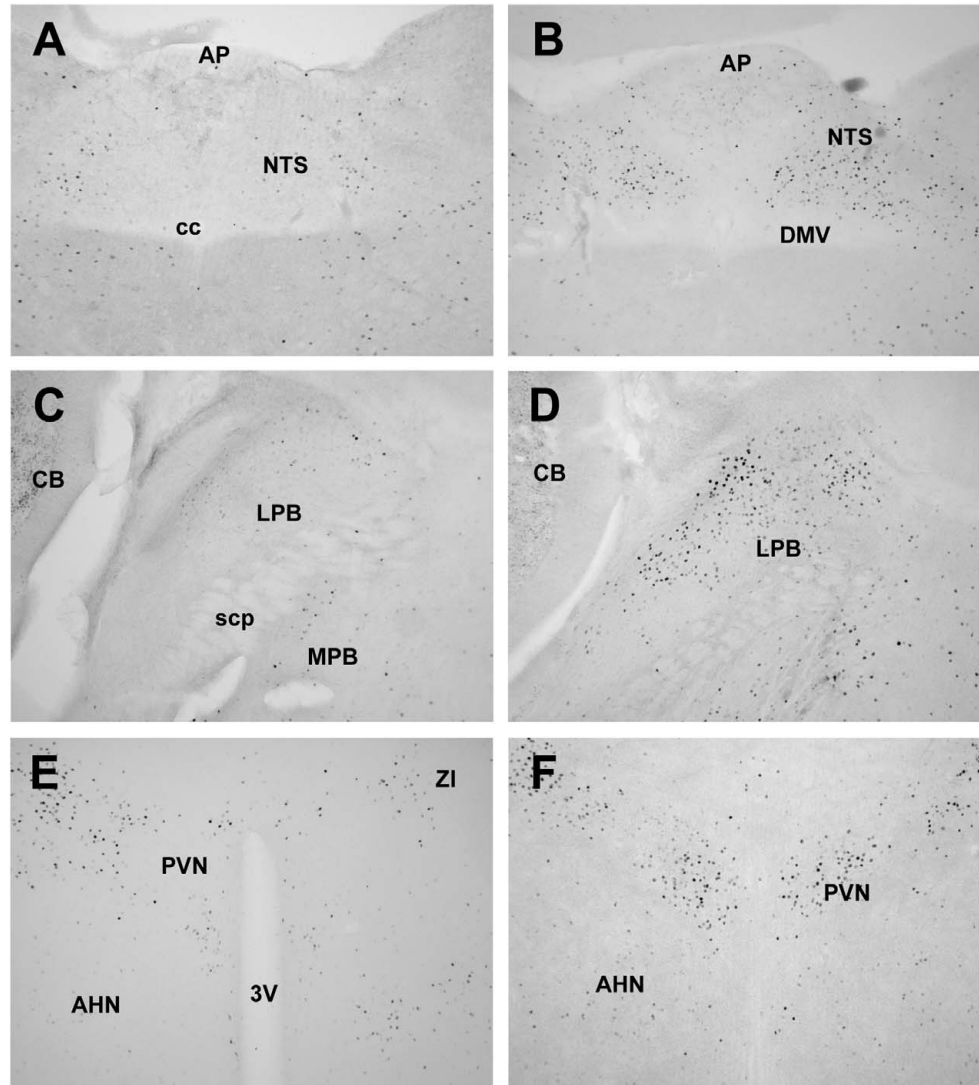
Bacteria in the gut are “seen” by the brain

- First “modern-era” demonstration of microbiota-gut-brain
- Introduction of novel bacterial species.
 - ▣ Critical that bacterial species chosen does not cause overt immune response or systemic infection
 - ▣ Use of live, replicating organism instead of killed or antigen
 - *Campylobacter jejuni* – infection/diarrhea not produced
- Natural infection route .
 - ▣ Per oral for *C. jejuni*
- Measure behavior.
 - ▣ Apparatus used in psychopharmacology
 - ▣ Anxiety-like behavior

Bacteria in gut induce anxiety-like behavior



Bacteria in gut can activate neurons in brain



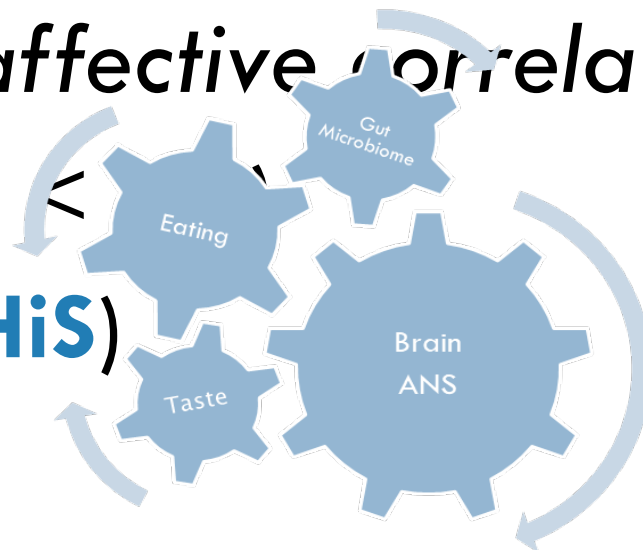
Microbiome and taste

□ Selective breeding on a taste phenotype

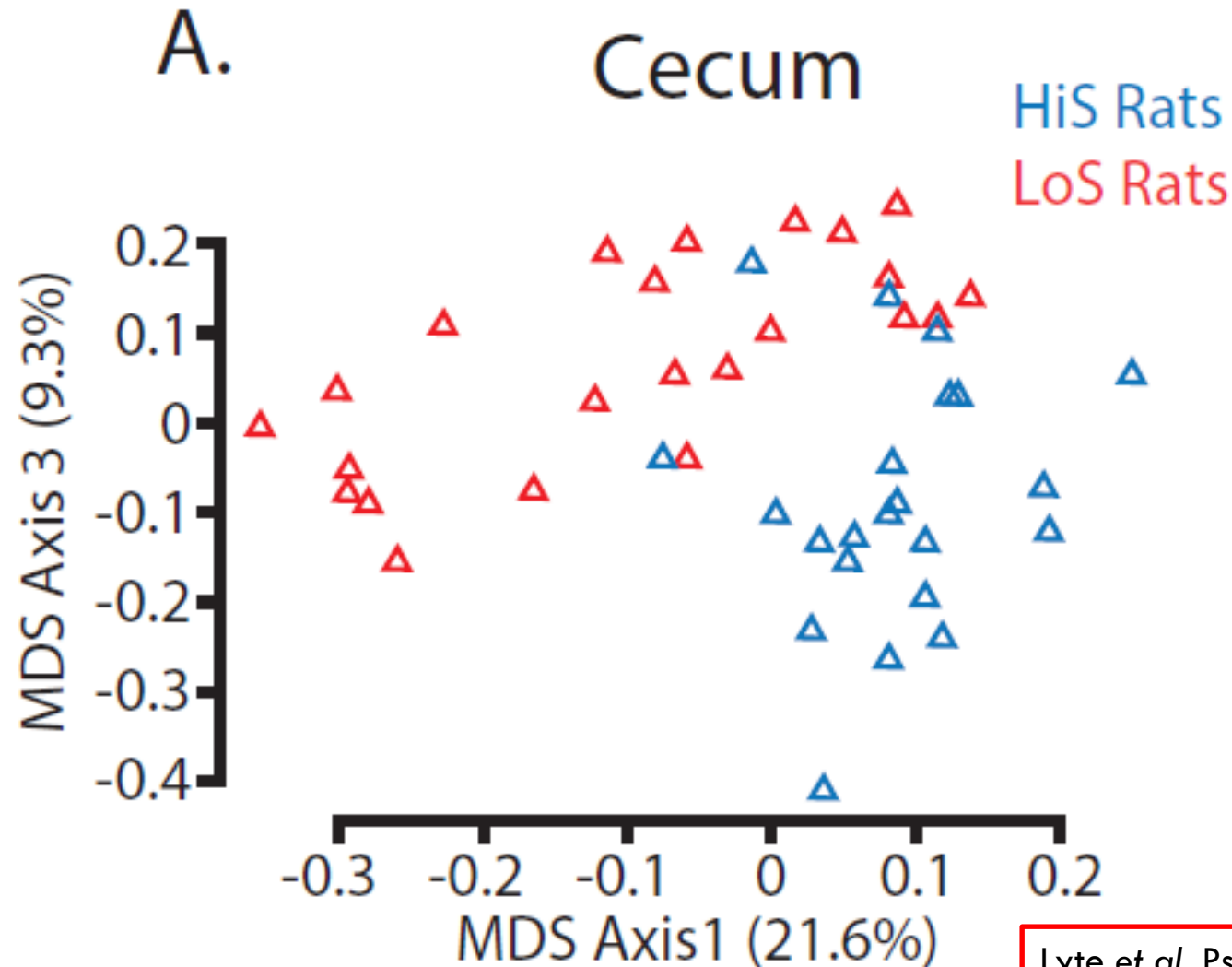
- Question addressed: Could gut microbes mediate line differences in affective processes?
- Low vs high saccharin intake (> 50 generations)
- Lines differentially consume most sweeteners
- First step: Do LoS and HiS rats host different microbial communities?
- Responsiveness to sweetness and bitter side tastes
- Consistency of taste aversion

□ Taste phenotype has affective correlates

- Reward sensitivity (**LoS** < **HiS**)
- Risk reactivity (**LoS** > **HiS**)



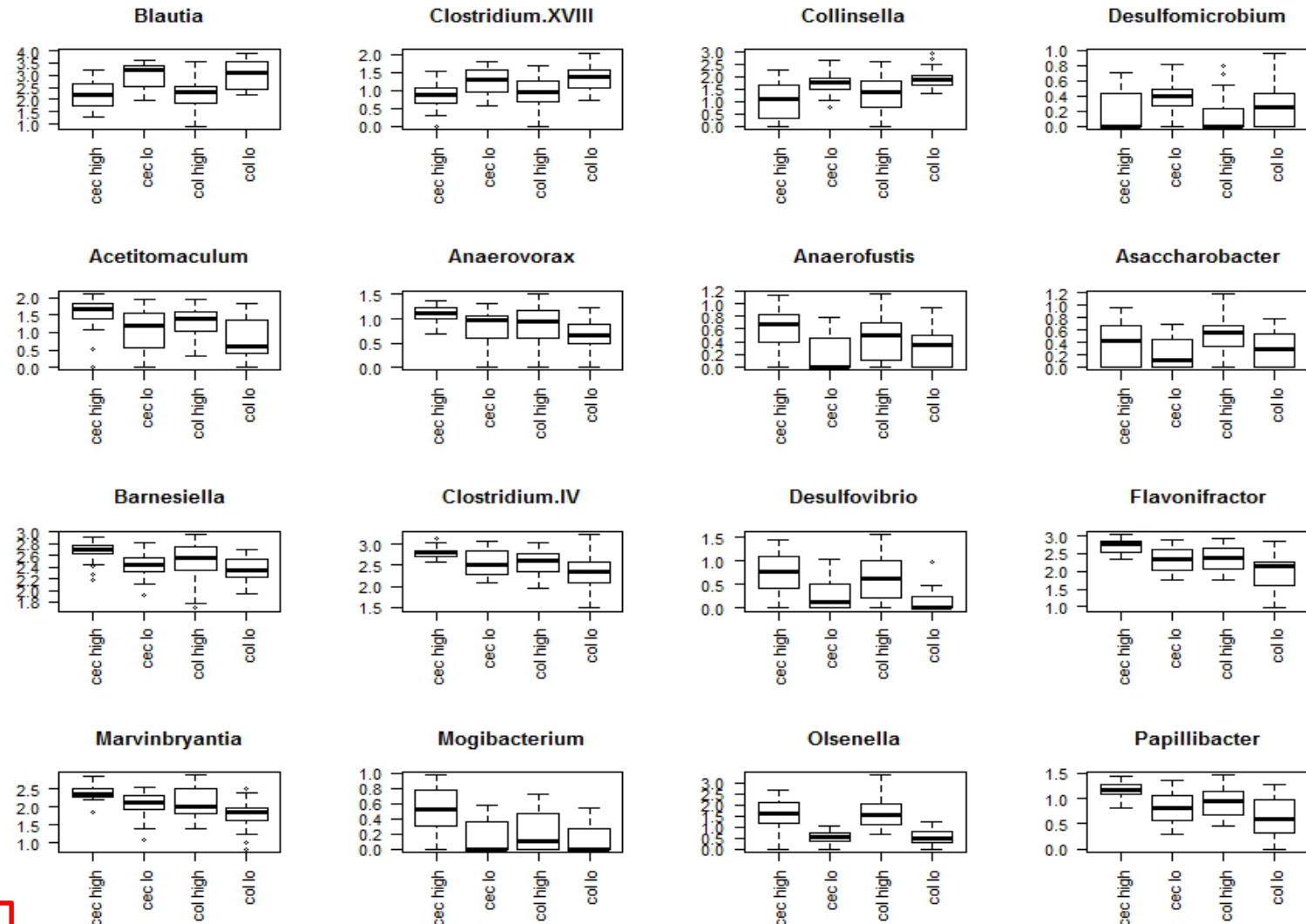
Microbial diversity differs between lines



Genus level differences

LoS > HiS

HiS > LoS



Overriding issues – Way forward for causation

- Studies shown **do not** prove causation, only correlation of microbes in sensory nutrition.
 - ▣ Mechanism, mechanism, mechanism - without it we will be groping in darkness essentially going from going from one promising mouse or human study to another.
 - ▣ Needed for design of intervention that meets EBM requirements.
- Combination of old school microbiology with study of nutrition based on robust literature with *in vitro* design of nutritional foods and pharmacology.
 - ▣ Mechanism examined proposed must at some level include neurochemistry.
 - ▣ Bioinformatics in the absence of culture approaches will not be enough.
- Use of microbial endocrinology as an evolutionary-based mechanism.
 - ▣ Almost certainly not the only mechanism.

Rationale behind development of new *ex vivo* methodology to examine microbial endocrinology



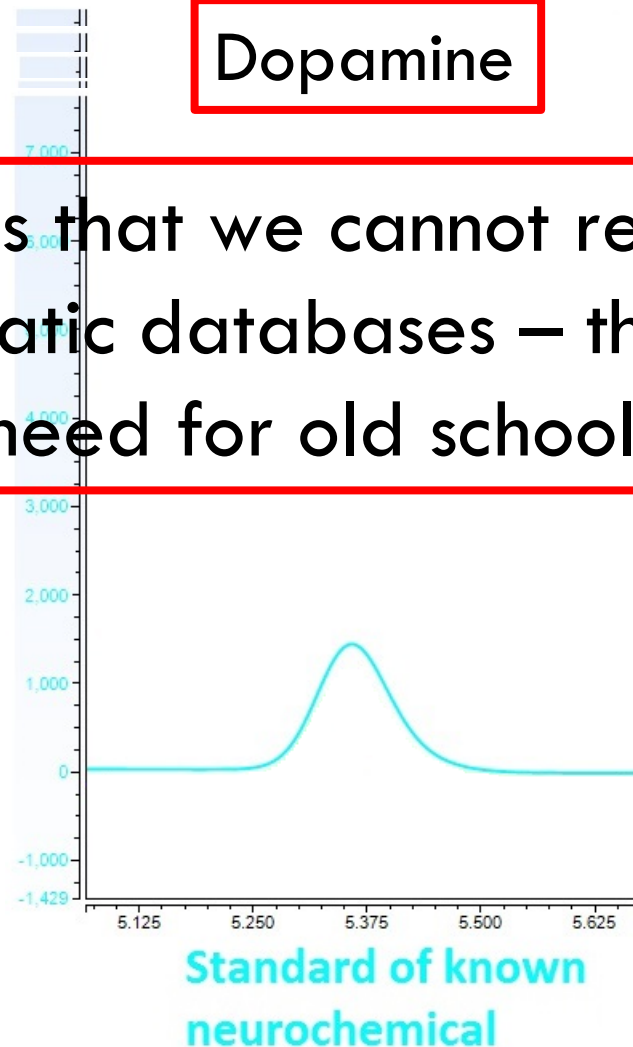
Potential for biofilm development



Neurochemical production is dependent on medium

Dopamine

This means that we cannot rely solely on bioinformatic databases – there is an absolute need for old school microbiology.



Control LB

Control MRS

Control sSIM

ML1082 in LB

ML1082 in MRS

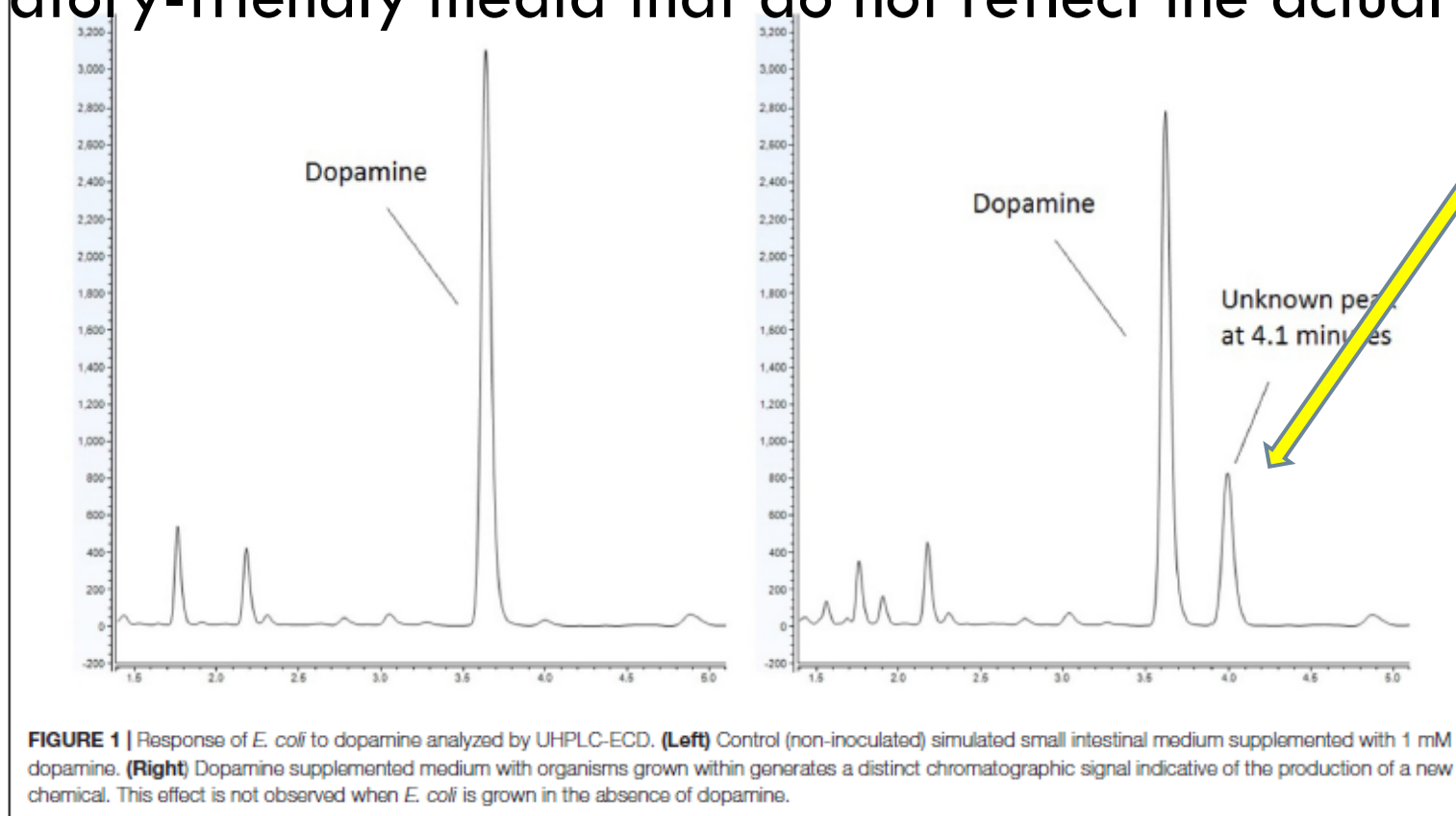
Standard

ML1082 in sSIM

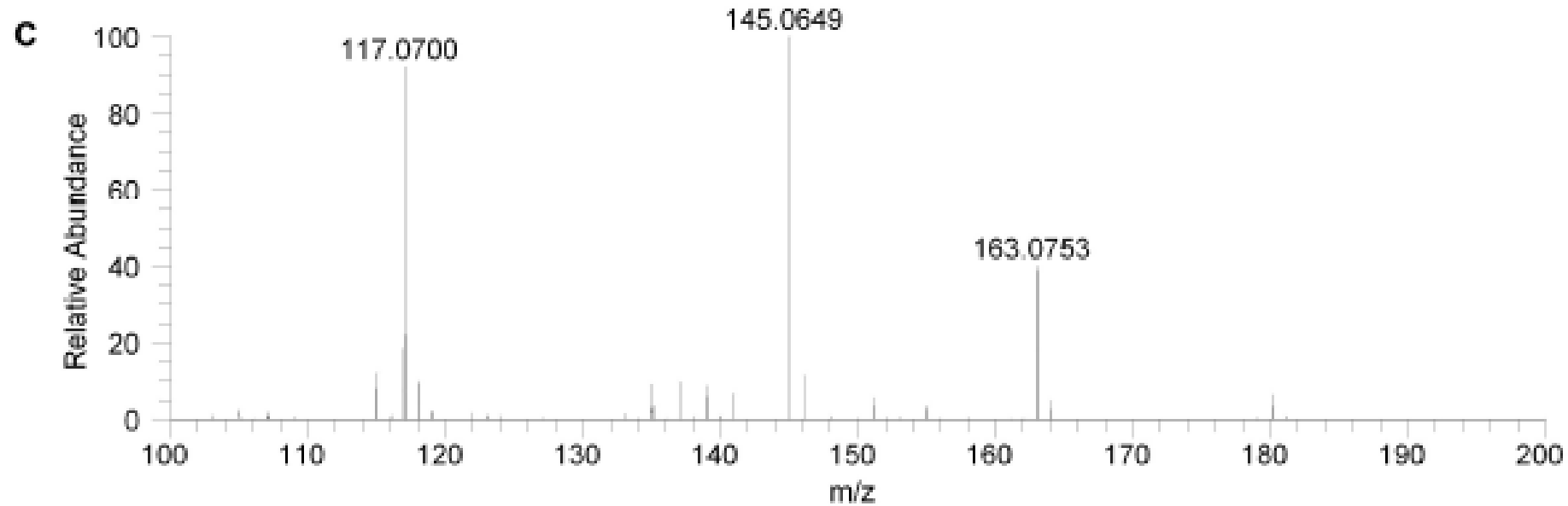
This is not due to the amount of L-dopa precursor.

Utility of an environmental-centric approach

- Databases largely contain information obtained from growth of bacteria in laboratory-friendly media that do not reflect the actual *in vivo* milieu.



What can then be discovered...

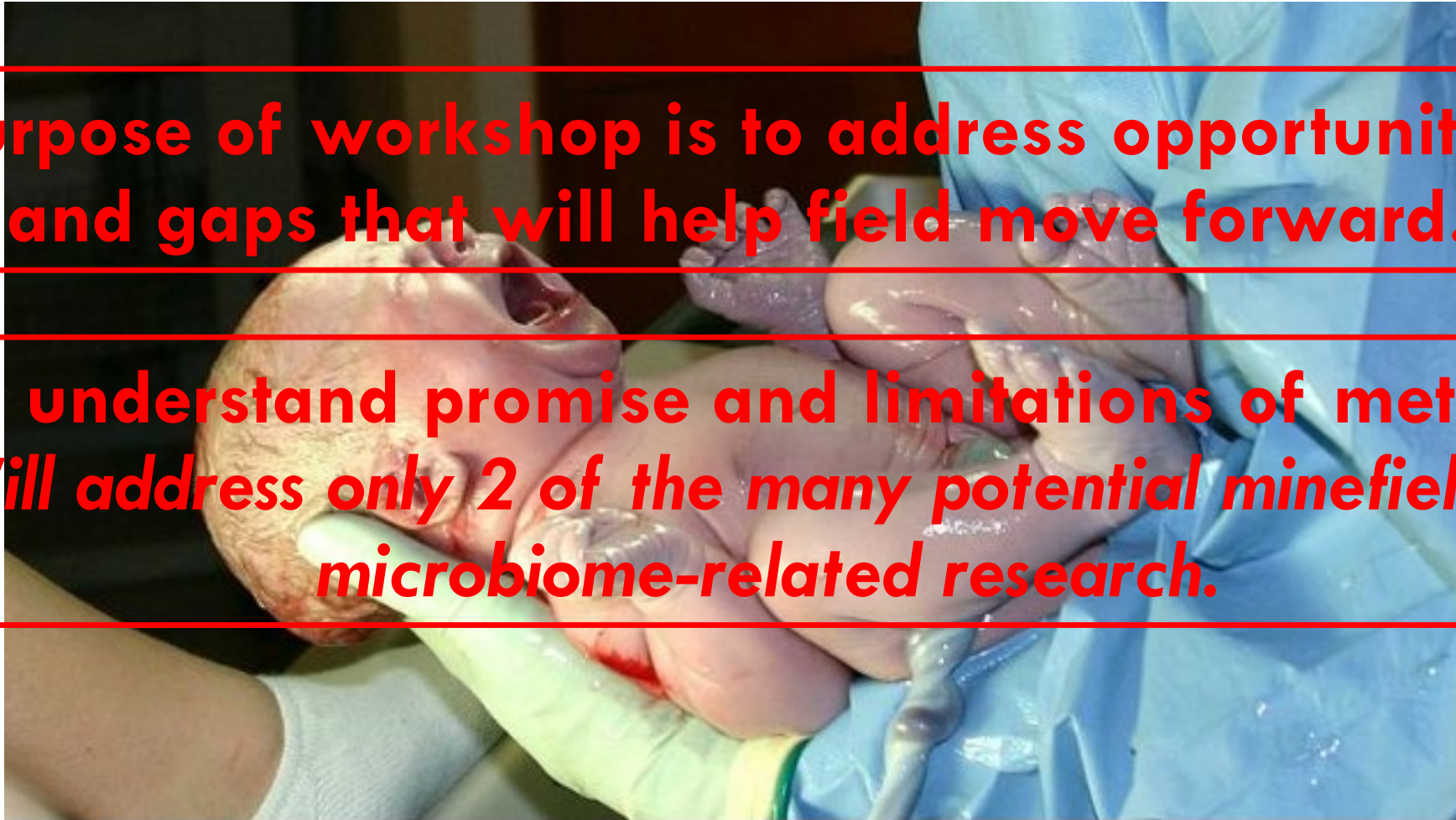


Peak is the neurotoxin salsolinol – first demonstration that growth of a normal gut bacterium can result in the production of a neurotoxin intimately involved in pathogenesis of neurodegenerative diseases such as Parkinson's disease.

Microbiota's purported role is ever expanding...

Purpose of workshop is to address opportunities and gaps that will help field move forward.

**Need to understand promise and limitations of methodology.
*Will address only 2 of the many potential minefields in microbiome-related research.***



State of microbiome and potential for influencing health



OPINION

Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet?

Eamonn M. M. Quigley

VOLUME 14 | MAY 2017 | **315**

Take home message of article

- We don't know what is normal
 - ▣ *“...we cannot and should not offer microbiota analysis as a diagnostic or prognostic tool in routine clinical practice.”*
- What microbiome-based treatments work?
 - ▣ *“Ultimately, a symptomatic response or cure to an intervention directed at the microbiome should clinch its role in a given disorder; to date, Clostridium difficile-related disease alone fulfils these criteria.”*

Gaps and opportunities

- Beginning of understanding microbiome-host interactions - Microbiome v2 – *Days of simple correlation are gone.*
 - ▣ What does this mean for assigning a role for the microbiome in sensory nutrition.
 - Design of experimental approaches and bioinformatic analyses employed will be critical if applications to human health will be realized.
 - Field is in constant flux – Just published that “...1,952 uncultured candidate species just identified...that encode hundreds of newly identified biosynthetic gene clusters”.
 - How many of these will be impacted by nutrition?
 - Almeida *et al* A new genomic blueprint of the human gut microbiota. Nature 568:499-506, 2019
- With this tsunami level amount of microbiome data it will be necessary to develop mechanistic-based approaches.
 - ▣ With advent of AI programs, will it be a data generating hypothesis?
 - ▣ Or more traditional approach?
 - Microbial endocrinology provides for an evolutionary-based approach that employs shared neurochemistry between host and microbe to examine mechanisms by which the gut microbiota, diet and host physiology interact as part of sensory nutrition.
 - ▣ Improve health and prevent infectious disease.
- Whatever route chosen, collaborations will be essential for the field to progress.