The Gut Brain Connection

Diet, the Microbiome and Brain Function

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What is the microbiome?

• “The characteristic microbial community occupying a reasonably well defined habitat which has distinct physico-chemical properties…encompasses their theatre of activities” – M. N. Burge, 1988 “Fungi in Biological Control Systems”

• “biome” – community

• “ome” – genome

• National Institutes of Health (NIH) established the Human Microbiome Project in 2008 to characterize microbial communities found at multiple human body sites and to look for correlations between changes in the microbiome and human health. http://hmpdacc.org
Objectives

- To know how gut microbiota may participate in bidirectional communication within the gut brain axis.
- To recognize factors that impact our gut microbiome.
- To become familiar with the pre-clinical data that suggests diet and the gut microbiome may play a role in mental illness.
“Let food be thy medicine and medicine be thy food.”

- Hippocrates
REVIEW

Gut Microbiota and Brain Function: An Evolving Field in Neuroscience

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Psychobiotics and the gut–brain axis: in the pursuit of happiness

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Abstract: The human intestine houses an astounding number and species of microorganisms, estimated at more than $10^{14}$ gut microbiota and composed of over a thousand species. An individual’s profile of microbiota is continually influenced by a variety of factors including but not limited to genetics, age, sex, diet, and lifestyle. Although each person’s microbial profile is...
Lost in Translation: The Gut Microbiota in Psychiatric Illness

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Let’s meet our 100 trillion friends

- 10x the number of human cells
- 150X the number of human genes
- 1000 species, 7000 strains
- Most bacteria live in our large intestine

Bacteria communicate with and affect our brain via:
- Products secreted into the bloodstream (5-HT, LPS, B12)
- Trigger cytokine production by gut mucosal immune cells
- Stimulating the vagal nerve through chemical messengers

Roles of the Microbiome

- Bacteria ferment polysaccharides in the proximal colon (no digestive enzymes there) into short chain fatty acids – supply 10% of our energy needs\(^1\)
- Control the population of pathogenic bacteria\(^2\)
- Main source of vitamin K, and to a lesser extent the vitamin B complex\(^2\)
- Help regulate our immune response\(^2\)
- Regulate tryptophan metabolism - impacts 5-HT and kynurenine levels – brain effects\(^3\)

Roles of the Microbiome

- Produce neurotransmitters:
  - *Lactobacillus* and *Bifidobacterium* → GABA
  - *E. coli*, *Bacillus* and *Saccharomyces* → norepinephrine
  - *Candida, Streptococcus, Escherichia* and *Enterococcus* → serotonin
  - *Bacillus* and *Serratia* → dopamine

- Affect brain development and plasticity by secreting BDNF, synaptophysin, post-synaptic density-95
Roles of the Microbiome

- Mice born in germ free conditions (no gut microbes) have\(^1\):
  - Altered expression and turnover of neurotransmitters in the enteric and central nervous system (5-HT and BDNF in the hippocampus). Introducing bacteria into these animals can reverse these abnormalities
  - Increased stress response and ACTH/cortisol levels. Bacterial colonization of the gut can reverse these abnormalities but only in very young mice.

This suggests there is a critical period during which the plasticity of neural regulation is sensitive to input from microbiota

Figure 2. Factors affecting gut microbiome. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Center for Medical Art & Photography © 2015. All rights reserved. CCF, Cleveland Clinic Foundation.
Impacts on the microbiome

- Mode of delivery: immediately after birth: Vaginal births – Lactobacillus (resemble mothers’ vagina), C-sections – Staphylococcus (resemble mother’s skin)¹

- Early nutrition and mode of delivery: Sampled babies stool at 4 months: Formula fed – increased *Clostridium difficile*, C-section – decreased diversity²

- Early life stress (maternal separation) in rhesus monkeys and rats affects composition of gut microbiota³

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Diet impacts the microbiome

- African children (vegetable fiber) – *Actinobacteria, Prevotella* and *Bacteroidetes*, European children (starch and protein) – *Firmicutes* and *Proteobacteria*. *Prevotella* produce high levels of SCFAs and have a protective role against gut inflammation.

- Adding plant fiber can shift microbiota but shift depends on initial microbiota composition.

- Enhanced gut microbial diversity was correlated with increased exercise and dietary protein intake in athletes compared with size, age and gender-matched nonathletic control groups. Athletes also exhibited lower inflammatory markers and improved metabolic markers.

Chemicals impact the microbiome

- Pharmaceuticals:
  - Antibiotics can have lasting impacts in decreasing diversity\(^1\)
    - *Bifidobacteria* and *Bacteroides* are esp. sensitive
  - Olanzapine: 21 days decreases *proteobacteria* and *actinobacteria* and increases *firmicutes*\(^2\)

- Pesticides:
  - Glyphosate (Round up – applied to wheat) known to affect microbiome of poultry, cattle and swine: increases pathogenic bacteria, *Salmonella* and *Clostridium* and decreases beneficial bacteria, *Lactobacillus, Bifidobacterium* and *Enterococcus*\(^3\)
  - Can glyphosate affect the human microbiome?

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Figure 4. Pathways involved in bidirectional communication between the gut microbiota and the brain. Multiple pathways exist through which the gut microbiota can modulate the gut–brain axis. They include endocrine (cortisol), immune (cytokines), and neural (vagus and enteric nervous system) pathways. The brain recruits these same mechanisms to influence the composition of the gut microbiota, for example, under conditions of stress. The hypothalamus–pituitary–adrenal axis regulates cortisol secretion, and cortisol can affect immune cells, alter gut permeability and barrier function, and change gut microbiota composition. Conversely, the gut microbiota and probiotic agents can alter the levels of circulating cytokines, and this can have a marked effect on brain function. Both the vagus nerve and modulation of systemic tryptophan levels are strongly implicated in relaying the influence of the gut microbiota to the brain. In addition, short-chain fatty acids (SCFAs) are neuroactive bacterial metabolites of dietary fibers that can also modulate brain and behavior. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor. From Cryan and Dinan.57
What mental illnesses could the gut microbiome affect?

- Preclinical and initial clinical research supports the role of the microbiome in the following illnesses:
  - Autism
  - Depression
  - Schizophrenia
  - Anxiety
  - ADHD
  - Dementia’s (AD, PD)
Autism

- C-sections, hospitalization, early infections, and associated antibiotic exposure are risk factors for ASD\(^1\)
  - These alter the developing gut microbiome\(^2\)

- NIH study 2012 (n=121) – 85% of children with autism have constipation, 92% report GI distress.\(^3\)

- Autism onset sometimes follows prolonged antibiotic usage (which, for some antibiotics, is known to result in the emergence of *Clostridium* strains)\(^4\)

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Autism

- Molecular-based analysis of stool samples found *Desulfovibrio* and *Clostridial* spp. more frequently in children with regressive autism than in healthy controls.¹
  - Note that anxiety and peculiar dietary habits associated with autism may account for microbiome shifts.

- Oral vancomycin (antibiotic not absorbed from the GI tract) administration in children with regressive autism showed short-term benefit in 8/10 with blinded raters²

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Autism- GI permeability

- Study of 90 children with autism, 146 first degree relatives and 64 control children, 146 control adults\(^1\):
  - Intestinal permeability (measured by urinary excretion of metabolically inert sugars after oral dosing) was abnormal in autistic children (36.7\%) and 1\(^{st}\) degree relatives (21.2\%) vs. controls (4.8\%)
  - Fecal calprotectin (measure of GI inflammation) was elevated in children with autism (24.4\%), relatives (11.6\%) and controls (0\%) (only those with abnormal IPT were tested– budgetary issue)

Autism

- Short chain fatty acids are metabolic products of gut bacteria when they ferment dietary fiber.
- 3 types made: acetic, propionic and butyric.
- can be excreted or absorbed to use as a source of energy. Butyric acid is an important source of energy for cells lining the colon. Different bacteria produce different SCFA.
- Propionic acid is a key fermentation product of Clostridia, Bacteroides, Desulfovibrio – bacterial species associated with autism\(^1\)
- intracerebroventricular administration of propionic acid, a microbial metabolite, produces autism-like behaviour in rats (repetitive behaviours, hyperactivity, anxiety and decreased socialization)\(^2\)

Autism

- Increased mean levels of propionic acid in stool of ASD children have been shown\(^1\).

- Lipopolysaccharide (outer cell wall component of gram negative bacteria) significantly higher in blood of adults patients with severe autism compared to healthy controls and inversely predicted scores on socialization domains\(^2\)

Autism

- **Mouse model of autism** – Maternal Immune Activation (inject pregnant mothers with the viral mimic poly (I:C)) yields offspring with the core communicative, social, and stereotyped impairments similar to autism, as well as a common autism neuropathology—a localized deficiency in cerebellar Purkinje cells
  - 2013 study\(^1\) showed increased intestinal permeability and cytokine levels and microbiome shifts similar to humans with autism in the adult MIA offspring vs. controls
  - Oral treatment of MIA offspring with *Bacteroides fragilis* corrected gut permeability, restored microbial composition, and improved communicative, stereotypic, anxiety-like and sensorimotor behaviors but retained deficits in sociability.

Autism: Clinical studies

- 12 week double-blind, placebo-controlled study of *Lactobacillus plantarum* given to children with autism resulted in:
  - significant increases in beneficial bacteria *lactobacilli* and *enterococci*
  - significant reduction in *Clostridium*
  - reduced GI problems
  - improved behaviour scores

- Gluten-free/casein-free diet (GF/CF) studies yield mixed results although some that have shown benefit have also have shown reduced GI permeability

2) Mayer at al. Bioessays 36: 933–939,
Autism: Summary of the research

- Risk factors for autism also known to impact the gut microbiome

- People with autism have altered microbiome (Clostridium, Desulfovibrio), high rates of GI complaints and increased GI permeability

- Administration of propionic acid (metabolite of Clostridium) into brains of mice induces autistic behaviour and high levels of propionic acid found in stool of people with autism

- Probiotics can improve autism behaviours in a mouse model and small human RCT
Depression

- In double-blind, randomized crossover trials administration of LPS to healthy humans:
  - transiently induced increased cytokine and cortisol secretion, anxiety and depressed mood and decreased verbal and nonverbal memory. Levels of cytokines positively correlated with impacts on anxiety, mood and memory\(^1\)
  - Similar results and increased visceral pain sensitivity\(^2\)
  - Increased activation of the right inferior orbital frontal cortex in response to emotional visual stimuli\(^3\)
  - pretreatment with citalopram but not bupropion, decreased LPS-induced anhedonia and fatigue but cytokine levels were unchanged\(^4\).

1) Reichenberg et al. *Arch Gen Psychiatry* 2001, 58:445–452;  
3) Kullmann et al. *Hum Brain Mapp* 2012. doi:10.1002/ hbm.22063;  
4) Dellagioia et al. *Brain Behav Immun* 2013;31: 197-204
Depression

- **Mice:** previous experience with physical and/or psychological stress (e.g. tail shock, social defeat) leads to an even more pronounced inflammatory cytokine release subsequent to LPS administration\(^1\)

- **Rats:** non-desirable gut microbes (e.g. *Campylobacter jejuni*), at quantities too low to produce a detectable immune response, influenced animal behavior indicative of human anxiety\(^2\)

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Depression

- In 1641 people referred to an internal medicine clinic for GI complaints, 84.1% state anxiety, 67% trait anxiety and 27% depressive symptoms\(^1\)

- DNA sequencing of fecal samples from 55 depressed and non-depressed people showed that Bacteroidales was overrepresented \((p = 0.05)\), Lachnospiraceae was underrepresented \((p = 0.02)\) in group with depression\(^2\)

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Depression

- Bifidobacterium can
  - inhibit LPS-induced inflammation, by blocking NF-κB activation in intestinal epithelial cell lines\(^1\)
  - Reverse elevated HPA axis response, cytokines and depressive symptoms in rats separated from mothers at birth\(^2\)

- In a RCT of healthy women, the group that consumed a fermented milk product for 4 weeks showed reduced reactivity to a task probing attention to negative context on fMRI
  - Changes seen in brain regions that control central processing of emotion and sensation\(^3\)

3) Tillisch et al. *Gastroenterology* 2013;144:1394–1401;
Depression

- In double-blind RCTs with healthy people:
  - People who were given a mixture of probiotics, containing *L. helveticus* and *B. longum*, for 30 days demonstrated significantly less psychological distress than their matched placebo counterparts\(^1\)
  - Healthy subjects who scored in the lower third for depressed mood showed significant improvement, after being fed a probiotic-containing milk drink for 3 weeks, as compared to their placebo counterparts\(^2\)

- The Mediterranean diet (increased fruits, vegetables and fish, decreased meat and sugars/starch) has shown benefit in reducing risk of depression\(^3\)
  - Could some of the impact be on dietary influence on the microbiome?

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Depression – Summary of the research

- People with depression have altered microbiomes and increased rates of GI complaints.
- Bacterial products (LPS) can induce depressive symptoms in humans that can be prevented by pretreatment with antidepressants and probiotics.
- Probiotics in rats can reverse the HPA and cytokine abnormalities and depressive symptoms induced by maternal separation.
- Probiotics in human show benefit in mood, distress and reactivity to negative stimuli.
Where to go from here?

- Anecdotal reports of recoveries from autism with the GAPS diet and fecal microbial transplants and recoveries from depression with dietary changes plus probiotics but clinical trials are needed to address these questions:
  - Can fecal microbial transplants and dietary and probiotic interventions be useful in clinical populations?
  - Can biomarkers of bacterial metabolites, inflammation and food antigens be used to select individuals that will benefit from dietary and probiotic interventions?
Summary

• The microbiome plays a role in the bidirectional gut-brain axis through bacterial metabolites, immune cells, the vagus nerve and the HPA axis.

• Preclinical data suggests that the gut microbiome and diet may play a role in the pathogenesis of several psychiatric illnesses including autism, depression and schizophrenia.

• Clinical trials are needed to evaluate whether fecal microbial transplant, dietary inventions and probiotics are viable therapeutic options.