It Takes Guts to Grow a Brain

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The Versatile Intestine: The intestine is not only a digestive-absorptive organ

- Largest immune organ of the body.
- Harbors a huge microbial ecosystem.
- Harbors the enteric nervous system.
Overview

• Brain-gut-microbial (BGM) signaling---introduction.
• BGM signaling in developmental programming-”critical developmental windows” for BGM interaction
• BGM interactions in human disease states:
  – Anxiety and stress
  – Depression
  – Autism
• Factors linking the microbiome and CNS
• The Future
New Tools for Evaluation of Microbes: Culture versus Non-Culture

400 species

2000 species
Commensal Microbes: Beneficial Effects for the Host

- Nutrient metabolism, digestion and absorption
- Tissue development (e.g., maturation of host immune system)
- Resistance to colonization with pathogens
- Maintenance of intestinal “homeostasis”
- Development of the Brain?
Brain, Gut and Microbial Signaling

Adapted from Ryan and O’Mahoney, Neurogastroenterology and Motility, 2011
The Enteric Nervous System (ENS): Examples of Effects on the Gut

- Hirschprung’s Disease—heightens risk of enterocolitis.
- Intestinal neuronal dysplasia—causes megalocystis, megacolon, and malrotation.
- Gut motor dysfunction is associated with myelomeningocele.
- Congenital or acquired spinal cord diseases cause neurogenic intestinal obstruction.
How the CNS influences Microbiome

- Regulation of satiety via signaling peptides (e.g. PYY)
- Hypothalamic, pituitary, adrenal (HPA) axis signaling (stress responses)---corticoid effect on permeability, mast cell activation.
- Release of signaling molecules, cytokines, and anti-microbial peptides into the gut lumen by neurons, enteroendocrine cells, immune cells and Paneth cells at the direct or indirect command of the CNS.
Evidence that Intestinal Microbes Affect the Brain

- Vitamin B6 (produced by intestinal microbes): profound effects on neurological and behavioral disorders.
- Intestinal microbes affect immune tolerance, therefore have an influence on autoimmune diseases, e.g. multiple sclerosis.
- Studies in animals show influence of intestinal microbes on anxiety-related behavior.
- In autism, schizophrenia and other neural disorders there are reports of specific bacterial species in the intestine that are altered.
How Microbiome influences CNS functions

- Enteric nervous system (ENS) cells responding to microbial metabolites convey intestinal signals to the CNS
- Toll-like receptors expressed on enteric nervous cells respond to microbial components
- Metabolite production (serotonin, melatonin, gamma-aminobutyric acid (GABA), catecholamines, histamine and acetylcholine).
Early Intestinal Microbial Environment

Sensory inputs from mother alter neural Circuitry
Gene transcription Cell/synapse and send signals to the GI tract.

Health Outcomes
Maternal Separation: Rat Pups and Subsequent Intestinal Permeability

- Maternal deprivation performed daily for 3 consecutive hours.
- Procedure applied between days 2 and 14.
- Control pups were left undisturbed with their dams.

Barreau, F. Gut, 2004
Effect of separation on intestinal microbes

- Separation (MS) applied between days 4 and 19.
- Some treated with probiotic (Lactobacillus) and others without.
- Corticosterone levels measured day 20.
- Sacrificed at 70 days.
- Ussing Chamber permeability studies on intestine.

**Effect on Intestinal Microbes**

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The effect of maternal separation on colonic bacteria</th>
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<tbody>
<tr>
<td></td>
<td>Total organisms (CFU(×10^3)/g tissue)</td>
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<tr>
<td>NS</td>
<td>36.9 (15.4)</td>
</tr>
<tr>
<td>MS</td>
<td>146.0 (45.1)*</td>
</tr>
</tbody>
</table>

Maternal Separation, Permeability and Intestinal Microbes: Effect of Lactobacillus Probiotic

- Isc and HRP flux (permeability measures) were significantly higher in the colon of separated (MS) versus non-separated (NS) pups.
- There was increased adhesion/penetration of total bacteria in MS pups, but a significant reduction in Lactobacillus species.
- Probiotic administration ameliorated the MS-induced gut functional abnormalities and bacterial adhesion/penetration at both day 20 and 60, and reduced the elevated corticosterone levels at day 20.
Influence of gut bacteria on biochemistry and development of brain

- Comparison of mice lacking normal gut microbiota to mice with normal gut microflora:
  - behavior
  - brain chemistry
  - brain development
<table>
<thead>
<tr>
<th>Source</th>
<th>Experimental Design</th>
<th>CNS Effects</th>
<th>Implications of the Study</th>
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<tbody>
<tr>
<td>Sudo et al., 2004</td>
<td>GF mice vs GF mice with recolonized gut vs GF mice with probiotics</td>
<td>GF mice had and exaggerated stress response. GF mice with recolonized gut had less stress response, and GF mice treated with probiotics totally reversed the changes in stress response (to normal stress response).</td>
<td>Microbiota play an important role in the development of stress response. Early in life, there is a critical window when colonization of the gut should occur to ensure normal development of the HPA system. Normal microbial gut colonization affects development of neuronal circuits involved in motor control and anxiety behavior.</td>
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<td>Diaz Heijtz et al., 2011</td>
<td>GF mice, GF mice with early gut colonization, and SPF mice</td>
<td>GF mice display increased motor activity and reduced anxiety, compared with SPF mice with a normal gut microbiota. GF mice colonized early in life with normal microbiota display similar characteristics as SPF mice, including a decreased levels of synaptophysin and PSD-95.</td>
<td>Changes in microbiota can change motor activity and neurotrophins independently of the autonomic nervous system.</td>
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<td>Bercik et al., 2011</td>
<td>SPF mice and GF mice treated with nonabsorbable antibiotics for 7 d</td>
<td>SPF mice treated with antibiotics (with or without vagotomy) transiently altered their microbiota and had increased exploratory behavior and hippocampal expression of BDNF. Colonization of GF mice with microbiota increased exploratory behavior and BDNF levels in the hippocampus.</td>
<td>There is evidence of rapid and sustained gut-brain communication in response to changes in microbiota. Behavioral changes can be observed after changes in microbiota. Peripheral sensory neurons contribute an early signal to the brain regarding changes in the microbiota (such as new potential pathogens). Probiotic bacteria have the potential to modulate behavior with regard to anxiety and depression.</td>
</tr>
<tr>
<td>Lyte et al., 1998</td>
<td>Mice infected with Campylobacter jejuni vs mice infected with chronic Helicobacter pylori</td>
<td>Mice infected with acute subclinical C. jejuni had rapid activation of vagal pathways and anxiety-like behavior. Mice infected with H. pylori had abnormal feeding behavior.</td>
<td>Is it possible that a critical window may exist after which reconstitution of microbiota and the immune system does not normalize the behavioral phenotype?</td>
</tr>
<tr>
<td>Goehler et al., 2005</td>
<td>Mice infected with C. jejuni</td>
<td>Infection with C. jejuni activated neurons in the nucleus of the solitary tract, as well as in brain regions associated with primary viscera-sensory pathways and the central autonomic network.</td>
<td></td>
</tr>
<tr>
<td>Bravo et al., 2011</td>
<td>Mice receiving long-term treatment with Lactobacillus rhamnosus</td>
<td>Treatment with L. rhamnosus induced region-dependent alterations in GABA (B1b) mRNA in the brain of these mice compared with controls. Treatment with L. rhamnosus reduced levels of stress, anxiety, and depression.</td>
<td></td>
</tr>
<tr>
<td>Neufeld et al., 2011</td>
<td>GF mice vs SPF mice</td>
<td>GF mice had less anxiety-like behavior compared with conventionally reared SPF mice. Conventionalized adult GF mice failed to normalize their behavior.</td>
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</tbody>
</table>

Abbreviations: BDNF, brain-derived neurotrophic factor; CNS, central nervous system; GABA, γ-aminobutyric acid; GF, germ-free; HPA, hypothalamic-pituitary-adrenal; mRNA, messenger RNA; SPF, specific-pathogen-free.

*Changes in the environment where animals are reared change the animal’s microbiota. Experiments using GF mice (with no bacteria in their gastrointestinal tracts) and conventionalized mice (originally GF mice that have had bacteria reintroduced to their intestinal tracts) showed changes in brain development and animal behavior.
Effects of Restraint Stress on the HPA Germ Free and SPF mice

Sudo, et al.
J Physiol.
2004 Jul 1
;558(Pt 1):263-75
Effects of Restraint Stress and reconstitution of microbes on HPA at different ages

Sudo, et al.  
J Physiol.  
2004 Jul 1;558(Pt 1):263-75
Summary of Sudo Studies

• Plasma ACTH and corticosterone elevation in response to restraint stress was substantially higher in GF mice than in SPF mice.
• The enhanced HPA response of GF mice was partly corrected by reconstitution with SPF feces at an early stage, but not by any reconstitution exerted at a later stage.
• Thus, an early developmental stage is required for the HPA system to become fully susceptible to inhibitory neural regulation by microbes.
GF mice display increased spontaneous motor activity.

*Adult germ free mice were more active and engaged in more “risky” behavior than mice raised with normal microorganisms.

Heijtz R D et al. PNAS 2011;108:3047-3052
GF mice display increased spontaneous motor activity.

Open Field Testing for Mice

Heijtz R D et al. PNAS 2011;108:3047-3052

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GF mice show altered expression of anxiety and synaptic plasticity-related genes.

Heijtz R D et al. PNAS 2011;108:3047-3052
Critical Windows

- GF mice colonized at 3 weeks with SPF microbes exhibited adult behavior.
- But if colonized at 6 weeks, they did not show adult behavior.
- Suggests hypothesis that there are critical periods during which microbes can affect neurobehavioral development and that the gut microbiome exerts programming influences for adult behavioral responses.
Results Summary

• Mice raised in germ-free conditions have significantly increased motor activity and decreased anxiety as compared to mice bearing normally colonized intestines.

• These developmental deficits in the germ-free pups are completely normalized by re-colonization of gastrointestinal flora.
Results Summary

• This reversal is dependent upon the stage of brain development, because microbial colonization of germ-free adult mice fails to reverse the behavioral phenotype.

• Thus, there is a critical developmental window during which intestinal microbiota are critical to normal brain development.
Depression, Happiness and the Microbiome

- Male GF mice have a significant elevation in the hippocampal concentrations serotonin compared with conventionally colonized control animals.
- When the GF animals were colonized with bacteria prior to adulthood, the central nervous system changes, especially those related to serotonin, could not be reversed.
- This indicates a permanent imprinting of the effects of absence of gut flora on brain function.

Clark, et al. Molecular Psychiatry 18, 666-673 (June 2013)
Autism Spectrum Disorder (ASD)

- Autism spectrum disorder (ASD) is a range of developmental neuro-behavioral disorders characterized by impaired social interaction and communication.
- The Centers for Disease Control and Prevention (CDC) estimates that 1 in 68 children (or 14.7 per 1,000 eight-year-olds) in multiple communities in the United States has been identified with autism spectrum disorder (ASD).
- The incidence of autism spectrum disorder (ASD) is rising in prevalence worldwide.
Microbes Associated with Autism Spectrum Disorders

- Increased Clostridium spp. (culture based)
- Increased Desulfovibrio spp.
- Reductions in Bifidobacterium spp.
- Reductions in Akkermansia muciniphila.
- Reduction of the phylum Bacteroidetes.
- Increase in Suterrella.
- Increase in Prevotella.
Pediatric Diseases Associated With Barrier Dysfunction

Tight junctions, leaky intestines, and pediatric diseases.
Interepithelial Junction

(a) Tight junction
(b) Desmosome (anchoring junction)
(c) Gap junction (communicating junction)

Plasma membranes of adjacent cells
Space between cells

0.25 μm
1 μm
0.1 μm

(b) Courtesy of L. Orci and A. Perrelet, Freeze-Etch Histology (Heidelberg: Springer-Verlag, 1975.) ©1975 Springer-Verlag.
©Addison Wesley Longman, Inc.
The Tight Junction
Para-vs. Transcellular Permeability
Autism, Microbes and a Leaky Gut

- Altered microbial metabolites in the stools, urine and blood of individuals with autism.
- Mouse models of autism have GI barrier defects.
- This permeability defect can be corrected with the human commensal Bacteroides fragilis.
- This also ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors. These findings support a gut-microbiome-brain connection in ASD and identify a potential microbial therapy.

Composition of the gut microbiota in a neonate. The composition of the microbiota is affected by numerous external and internal factors, beginning at birth.
What Does this mean for the neonate and the neonatologist?

TABLE 2  Most Commonly Reported Medications

<table>
<thead>
<tr>
<th>Medication Name</th>
<th>Frequency, A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>186,799</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>171,388</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>90,152</td>
</tr>
<tr>
<td>Vitamin (multivitamin)</td>
<td>64,329</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>55,455</td>
</tr>
<tr>
<td>Caffeine citrate</td>
<td>48,814</td>
</tr>
<tr>
<td>Furosemide</td>
<td>47,278</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>44,218</td>
</tr>
<tr>
<td>Beractant (Survanta)</td>
<td>36,410</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>27,541</td>
</tr>
</tbody>
</table>
What Lies Ahead?

• With our refining knowledge of the microbiota—intestine---brain interactions, we will need to pay even closer attention to perturbations in the intestinal microbiota:
  – Antibiotics, probiotics, other forms of microbial interventions.
  – C section vs. vaginal delivery
  – Nutritional composition
  – Environment of the infant (maternal contact, intensive care interventions).
Take Home Messages

• Brain-Gut-Microbe signaling exists and the presence and composition of microbes play an important role.

• There are early life “critical windows” during which this signaling is important for brain development.
• Disorders such as autism, depression, and anxiety are linked to the intestinal microbiome.
• Mechanisms for these relationships are just beginning to be understood.
• Therapeutic interventions (antibiotics, probiotics, fecal transplantation) may be useful.