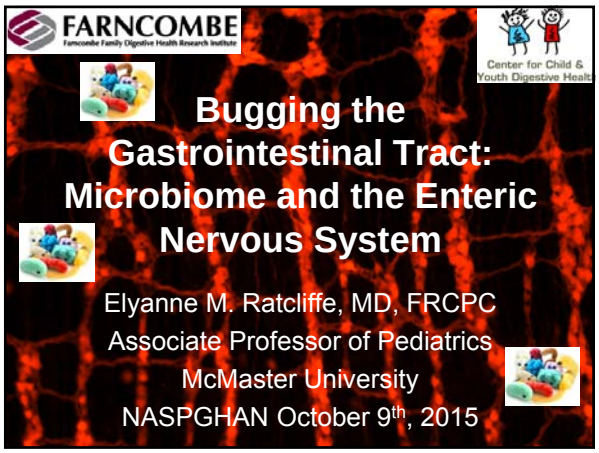


I have no financial relationships with a commercial entity to disclose.

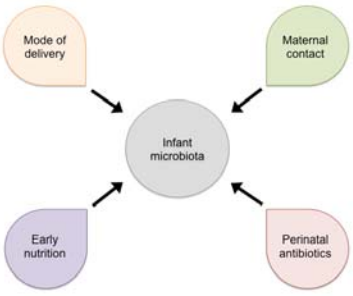
Learning Objectives

- Provide an overview of microbial colonization of the GI tract and development of the enteric nervous system
- Discuss examples of microbiota-enteric nervous system interactions
- Highlight potential implications in the context of early life influences on microbial colonization
- Discuss areas of potential research focus from both basic science and clinical perspectives

Pediatric disorders of GI motility are common…

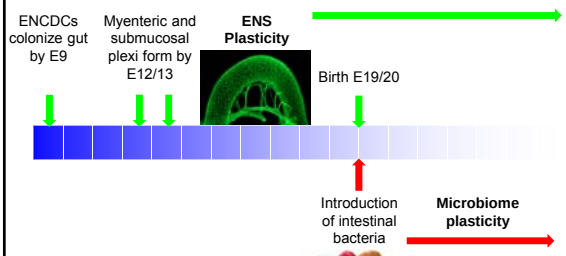
…but not well understood

A microbiome is born



Palmer PLoS Biol 2007
Penders Pediatrics 2006

The ENS continues to develop in early postnatal life



Schafer Anat Record1999
de Vries Am J Physiol 2010
Foong J Physiol 2012
Hooper Trends in Microbiol 2004

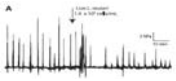
Bacteria can influence the ENS

Probiotic-induced changes in chemical coding of enteric neurons

- *Saccharomyces boulardii*
  - ↓ calbindin immunoreactive neurons
- *Pediococcus acidilactici*
  - ↑ galanin and CGRP immunoreactive neurons

Probiotic-induced changes in gut physiology

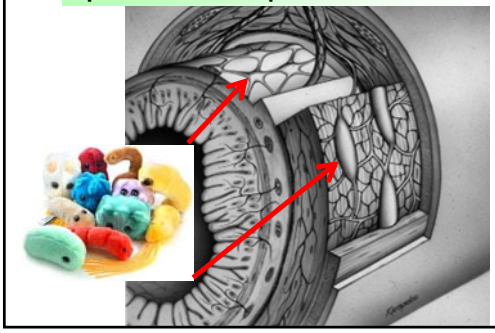
- *Lactobacillus reuteri* (JB-1)
  - ↓ amplitude of jejunal and colonic contractions
  - Mimicked by IK_{Ca} channel blocker
- *Bifidobacterium longum*
  - ↓ AH neuron excitability



Kamm Neurogastroenterol Motility 2004
Di Giancamillo Neurogastroenterol Motil 2010
Wang Neurogastroenterol Motil 2010
Wang FASEB 2010
Khoshdel Neurogastroenterol Motil 2013

HYPOTHESIS:

Intestinal microbiota can influence the postnatal development of the ENS



Axenic/Gnotobiotic Mouse Facility

- Specific pathogen-free (SPF) mice
- Germ-free (GF) mice
- Altered Schaedler Flora (ASF) mice
  - Standardized microbiota
  - 8 strains: 6 gram-positive and 2 gram-negative

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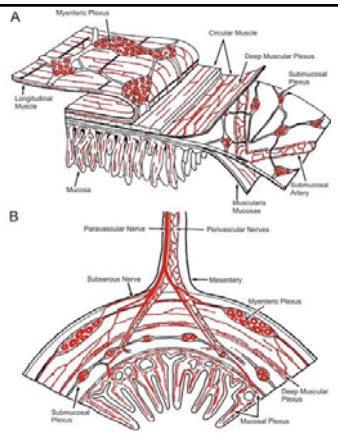
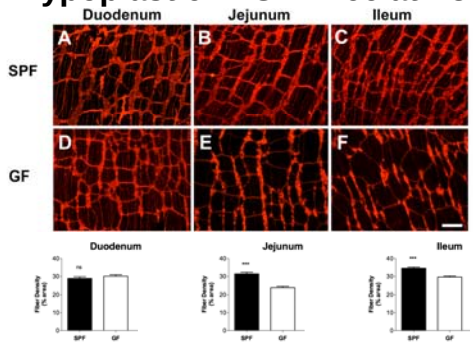


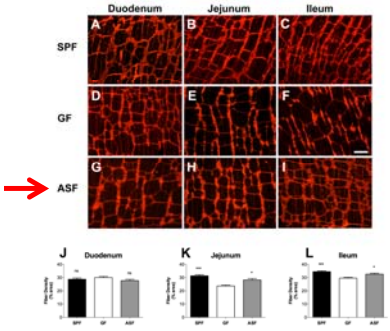
Furness

The myenteric plexus is hypoplastic in GF mice at P3



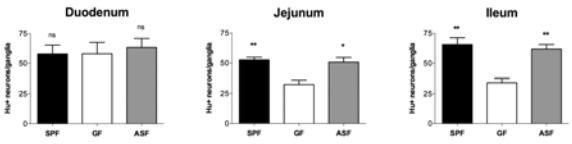
Collins Neurogastroenterol Motil 2013

Simplified flora sufficient for normal ENS formation



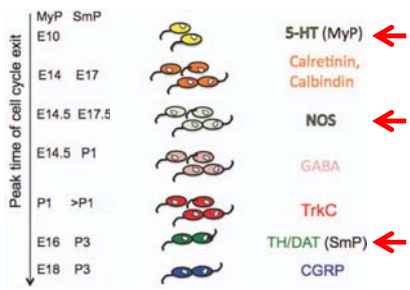
Collins Neurogastroenterol Motil 2014

Neuronal cell bodies per myenteric ganglion are decreased in GF mice at P3



Collins Neurogastroenterol Motil 2014

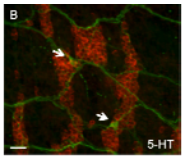
Which populations of neurons contribute to the abnormal ENS in GF mice?

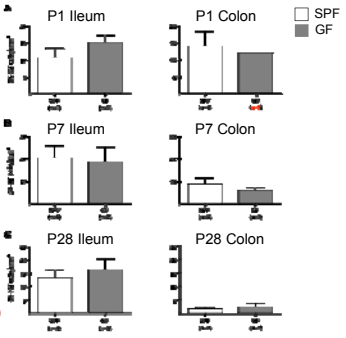


Chalazonitis Develop Neurobiol 2012

Serotonergic neurons

Number of 5-TH-positive myenteric neurons per square millimeter of tissue

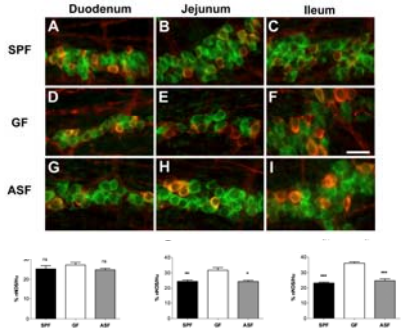




Human neuronal protein C/D (Hu)
5-hydroxytryptamine (5-HT)

Mungovan JPGN Abstract 2013

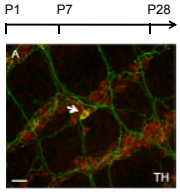
Nitrergic neurons are increased in GF mice at P3

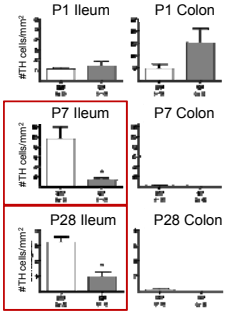


Collins Neurogastroenterol Motil 2014

Dopaminergic neurons are decreased in GF mice at P7 and P28

Number of TH-positive myenteric neurons per square millimeter of tissue. ($p < 0.05$)

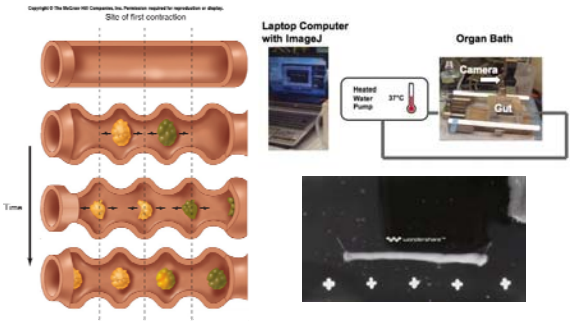




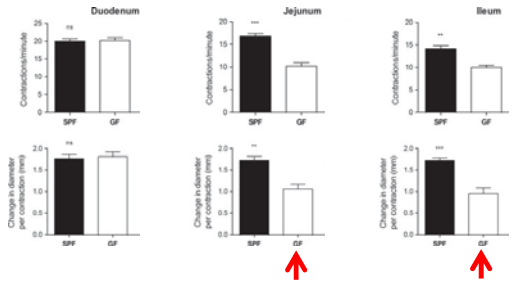
Human neuronal protein C/D (Hu)
Tyrosine hydroxylase (TH)

Mungovan JPGN Abstract

Spatiotemporal mapping

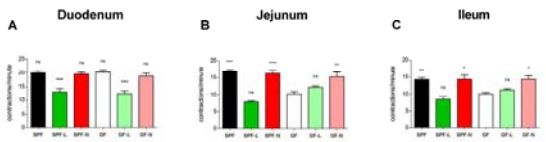


Intestinal contractions are decreased in GF mice at P3



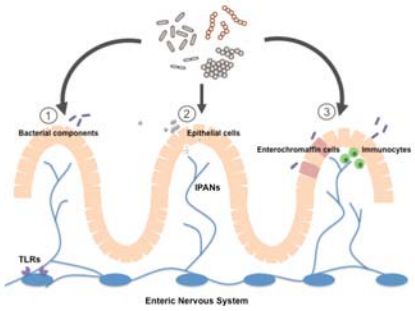
Collins Neurogastroenterol Motil 2014

Intestinal contractions are increased by nitrergic inhibition (NOLA)



Collins Neurogastroenterol Motil 2014

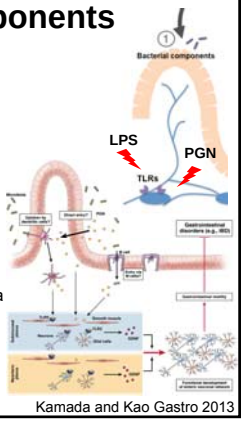
Potential mechanisms



Mungovan and Ratcliffe The Gut-Brain Axis *in press* 2015

Bacterial components

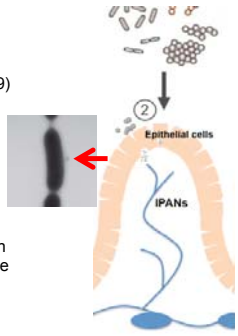
- Majority of emerging research has been identifying toll-like receptors (TLR) in mediating microbial-ENS interactions
- TLR4: Present in the ENS (postnatal and adult); Mice lacking TLR4 have an abnormal ENS; Exposure to LPS can stimulate enteric neurons (Rumio J Cell Physiol 2006; Anitha Gastro 2012)
- TLR2: Present in the ENS, enteric glia and intestinal smooth muscle; Mice lacking TLR2 have an abnormal ENS; ENS defects seem to be mediated by GDNF (Brun Gastro 2013)



Kamada and Kao Gastro 2013

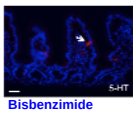
Epithelial cells

- Intestinal microbiota are necessary for the normal excitability of IPANs (MacVey Neufeld Neurogastroenterol Motil 2009)
- Microvesicles were formed from *Lactobacillus rhamnosus* (JB-1) and enriched for heat-shock protein components (Al-Nedawai FASEB J 2015)
  - Only produced functional effects on enteric neurons when applied to the epithelium
  - No effects when applied to enteric neurons directly

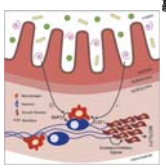


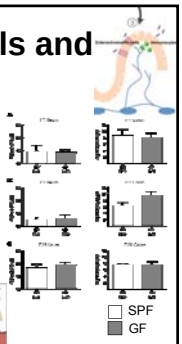
Enterochromaffin cells and immunocytes

- Interactions between bacteria and the ENS could also be mediated through:
  - Enterochromaffin cells (Rhee Nature Rev 2009); No significant difference in EC cells between GF and SPF mice (P1-P28; Mungovan JPGN abstract 2013)
  - Immune cells e.g. macrophages in the muscularis externa (Muller Cell 2014)



Bisbenzimide
5-HT



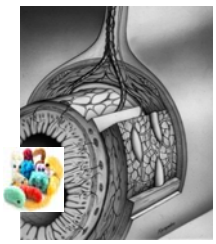


Clinical implications

- Microbiota might play a role in the pathophysiology of GI motility disorders:
  - Children exposed to antibiotics in early life have been found to have an increased incidence of abdominal pain (Uusijarvi Gastro 2012)
  - Altered stool microbiota profiles have been found in children with irritable bowel syndrome and with constipation (Rigsbee Am J Gastro 2012; Zoppi Acta Paediatr 1998)
- Probiotics have therapeutic potential:
  - Premature infants treated with *Lactobacillus reuteri* have a significant decrease in regurgitation and increase in the rate of gastric emptying (Indrio J Pediatr 2008)
  - Infants treated with *Lactobacillus reuteri* for constipation have a significant increase in frequency of bowel movements (Coccorullo J Pediatr 2010)

Conclusions

- Intestinal microbiota can influence the normal development of the enteric innervation
- Future studies are needed to investigate the potential mechanisms of microbial-ENS interactions
- Clinical studies linking clinical presentations of GI motility disorders with pathophysiological findings should consider including microbiota profiling



FARNCOMBE
Farncombe Family Digestive Health Research Institute

Hamilton Health Sciences

McMaster Children's Hospital

Center for Child & Youth Digestive Health

- Ratcliffe Lab
  - **Rajka Borojevic**
  - **Josh Collins**
  - **Kal Mungovan**
  - Jenna Dowhaniuk
  - Kate Prowse
  - Justin Brunet
  - Megan Wang
- Verdu Lab
  - AGU Staff
- Huizinga Lab
  - Sean Parsons
- Khan Lab
  - Janice Kim
  - Sharif Shajib

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