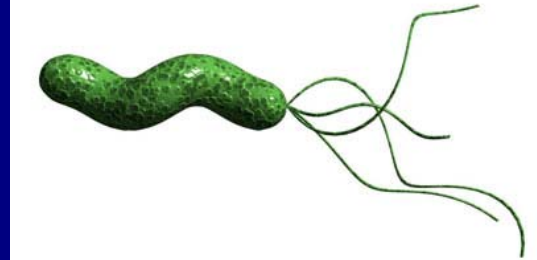


Figure 1 | Home for an abundant microbiological flora. The human gut and (inset) a scanning electron micrograph of part of the small intestine, with some bacterial inhabitants picked out in green.



Post DDW 2010: Latest insights into pathophysiology of FBD

Christopher Chang, MD, PhD

GI Motility Program

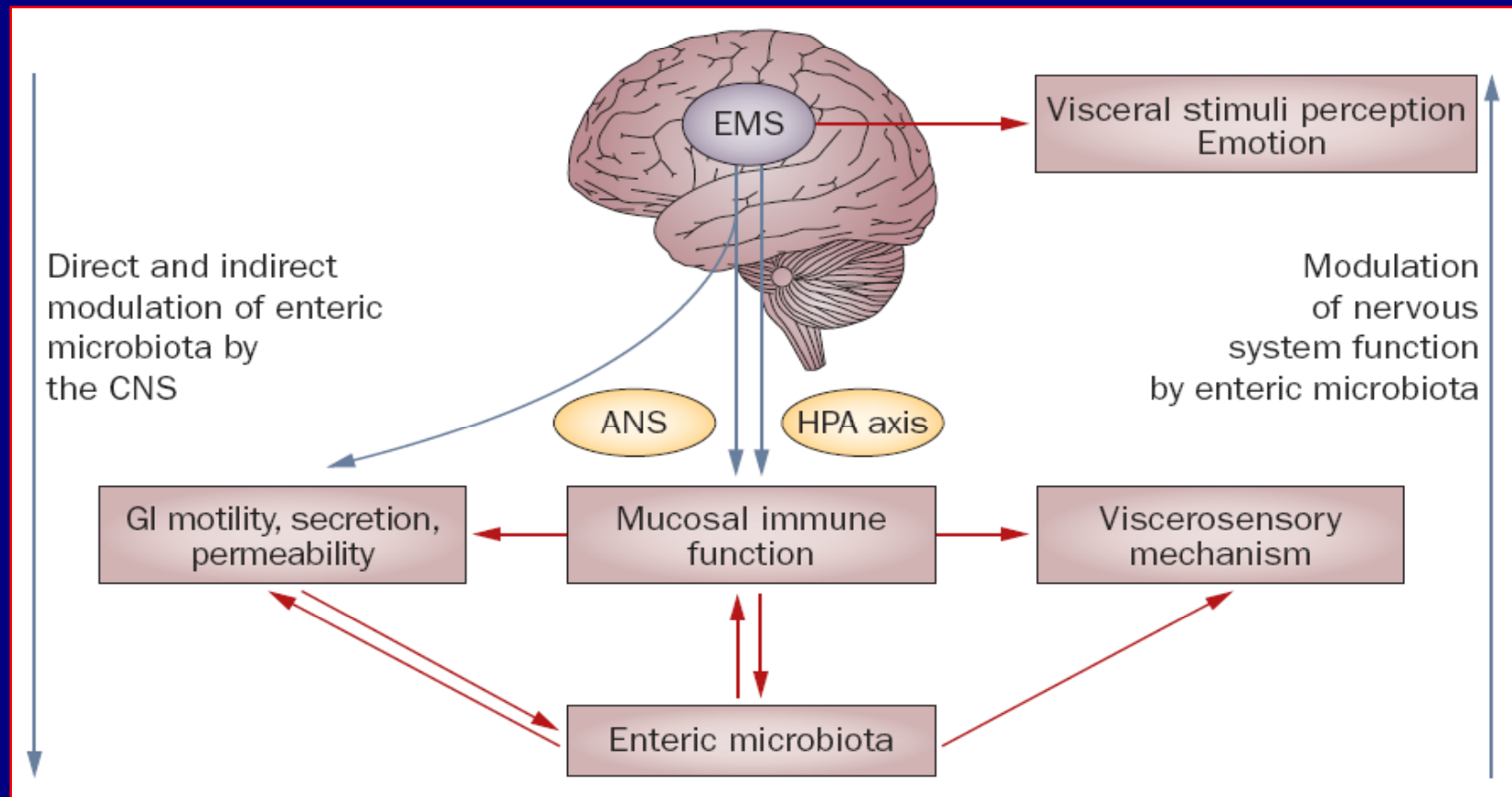
Cedars-Sinai Medical Center

&

Department of Microbiology, Immunology, and Molecular
Genetics

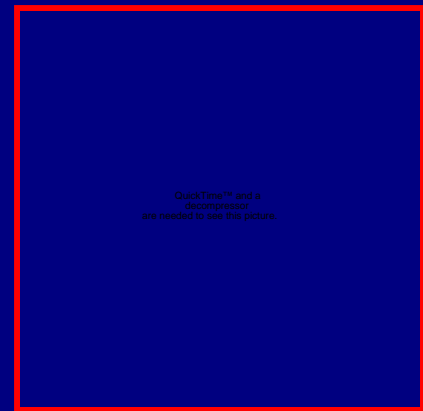
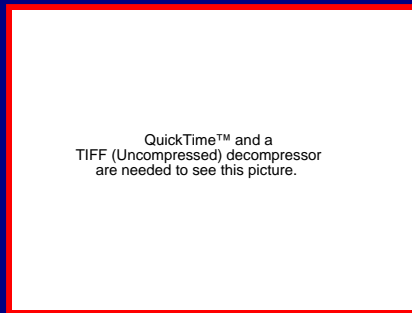
David Geffen School of Medicine at UCLA

Brain-gut-enteric microbiota axis



Overview

- Gut flora role in ENS and FBD #25, 965, 10
- Mechanistic insights #475j, 572, 670
 - Nerve growth in IBS
 - PI-IBS
- Quick shots # W1367, 29

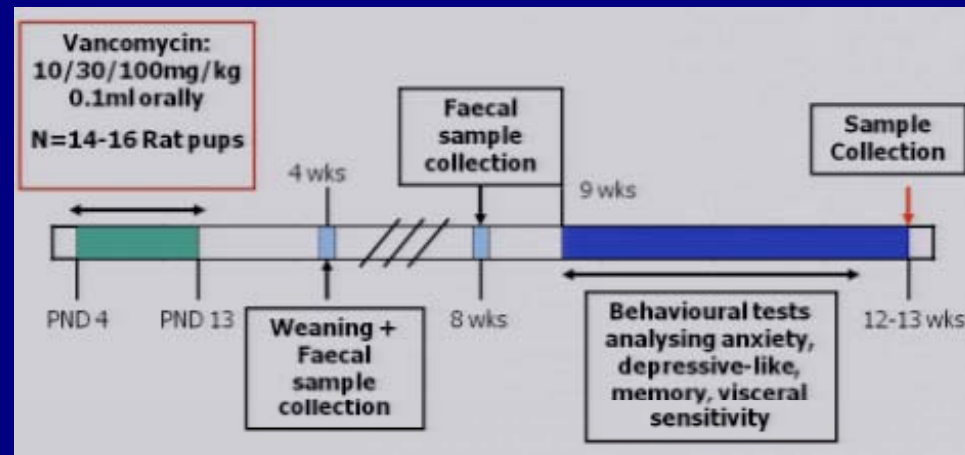


Early-life Dysbiosis Leads to Visceral Hypersensitivity in Adulthood (#25)

- Early post-natal period is most dynamic in establishing gut microbiota
- Antibiotic use can dramatically alter gut microbiota; effects are usually transient
- IBS patients have demonstrated differences in gut flora composition compared to healthy controls

Question: Will neonatal antibiotic administration cause long-lasting effects on brain-gut-microbiota axis?

Ear-life Dysbiosis Leads to Visceral Hypersensitivity in Adulthood (#25)



Findings:

- Transient flora changes at week 4 --> resolves by week 8
- No induction of anxious behavior (elevated plus maze)
- No induction of depressive-like behavior (open field arena)
- No impairment of spatial memory (water maze)
- Elevated plasma corticosterone, but not significant
- Increased % neutrophils in spleen
- No change in cytokine profiles in *in vitro* stimulated splenocytes or WBCs (e.g. IL-6,10, TNF_{α} , IFN_{γ})

Vancomycin induced dysbiosis leads to visceral hypersensitivity in adult rats

QuickTime™ and a
decompressor
are needed to see this picture.

QuickTime™ and a
decompressor
are needed to see this picture.

Dose response effect seen

Conclusions (Abstr#25)

QuickTime™ and a
decompressor
are needed to see this picture.

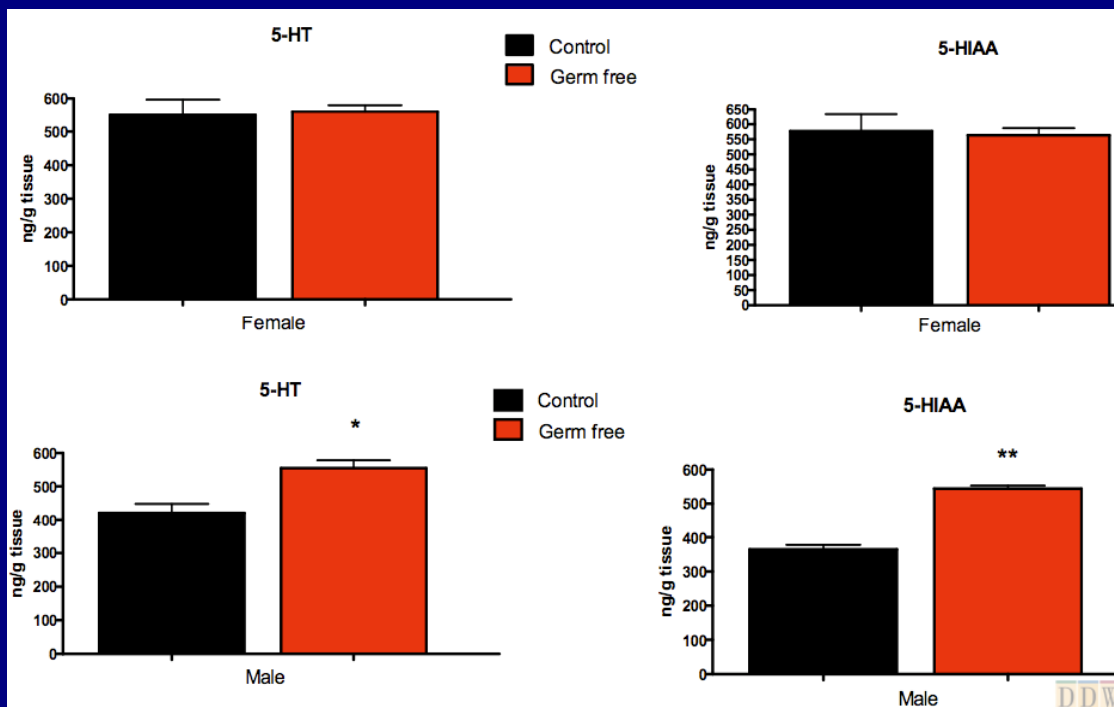
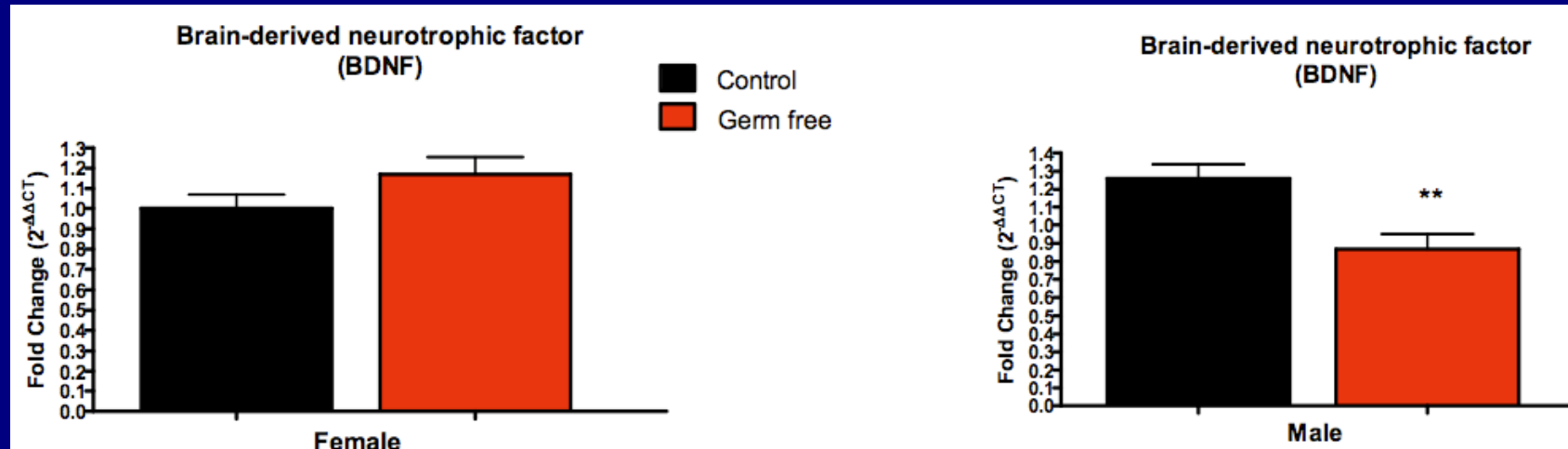
Comment

- Appropriate host-microbe interaction interrupted during critical time window?
- Intriguing study that raises more questions:
 - Changes in bowel frequency, weight?
 - Characteristics at other time points?
 - Inflammation and gut permeability changes?
 - More subtle microbiome differences in vanco-treated group?

The Brain-Gut-Microbiome Axis: Gender and Region-specific Effects on the Microbiome on Central Neurotransmitter and Neurotrophin Levels (#965)

- Why the interest?
 - Early life stresses can alter the gut microbiota (O'Mahony, '09)
 - Probiotics can alter tryptophan levels (Desbonnet '09)
 - Effect of germ-free environment on CNS?
- Brain derived neurotrophic factor (BDNF)
 - Neurotrophin supporting neuronal survival and growth
 - Important in enteric nervous system
 - Decreased BDNF in GF vs conventional animals
- The experiments:
 - GF vs conventionally colonized Swiss Webster mice sac'd at 6 wks
 - Brain tissue dissected -->NT and metabolites measured by HPLC and ECD; BDNF measured by qPCR

Gender differences in hippocampal BDNF and 5-HT



Findings are specific

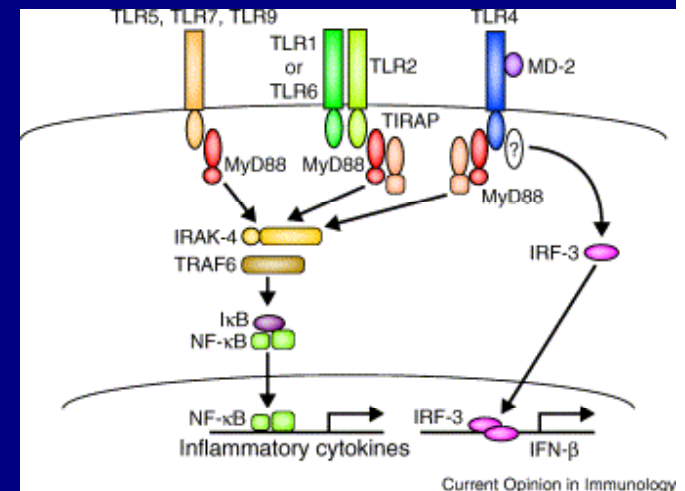
- No alteration in noradrenaline or dopamine in hippocampus
- No alteration in 5-HT of hypothalamus or prefrontal cortex

Bottom Line:

Gut microbiome influences CNS neurotransmission

- Affects BDNF expression
- Gender and region specific effects on serotonergic system
- Altered noradrenergic system

Altered GI motility and reduced nNOS neurons in TLR4 mutant mice (#10)



- Toll-like receptors (TLRs) recognize microbial patterns as part of the innate immune response
 - E.g. LPS (TLR4), bacterial flagellin (TLR5)
 - Signals via central adaptor molecule MyD88
 - Key pathways in mediating influence of microbes beyond immunity??
- TLR4 expressed in myenteric and submucosal plexuses of murine intestine and human ileum (Barajon, J Histochem Cytochem, 2009)
- Question: What is the role of TLR4 signaling on enteric neurons and GI motility?

Abstract #10 continued

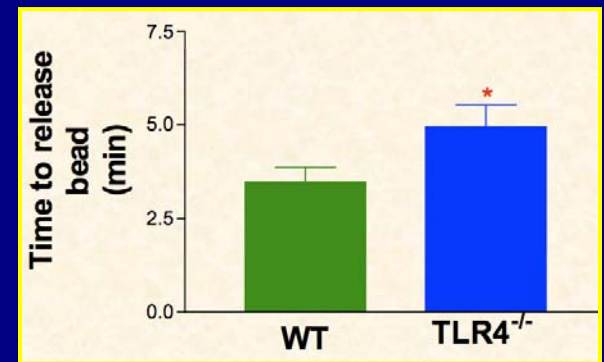
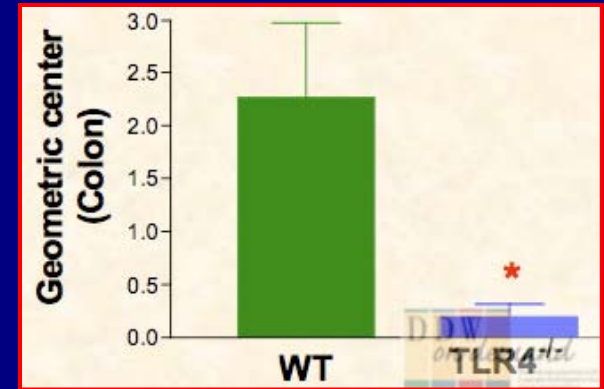
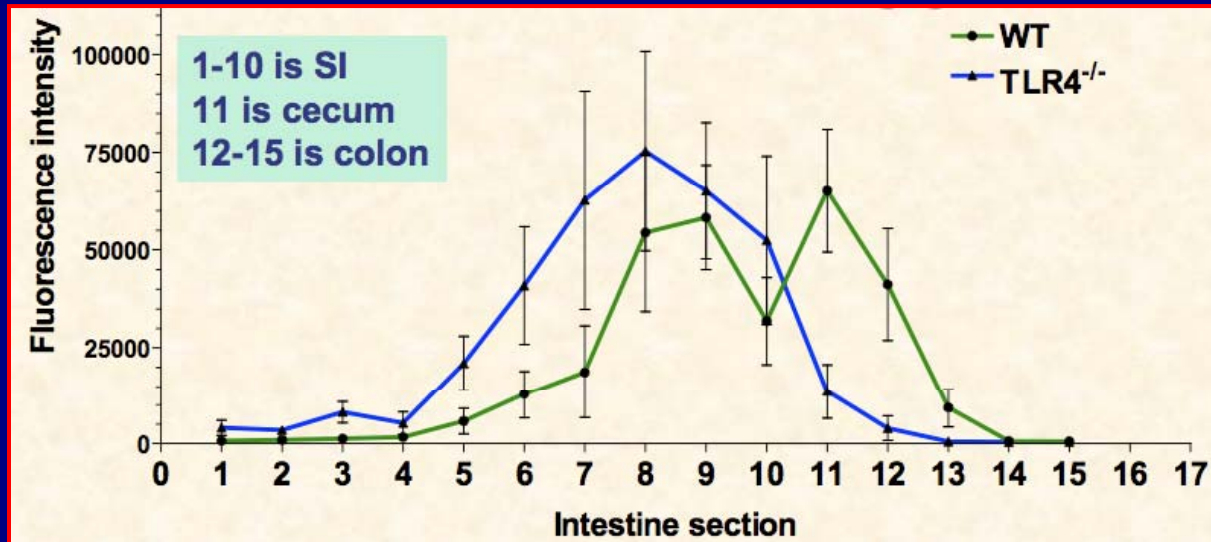
- **Methods**

- 17-18 week old TLR4 mutant, MyD88 KO, and wildtype GF mice
- Cholinergic and nitrenergic neurons assessed by
 - NADPH diaphorase, Achetylcholinesterase chemical staining
 - nNOS, TUJ1, and ChAT immuno staining
- GI motility testing

- **Results**

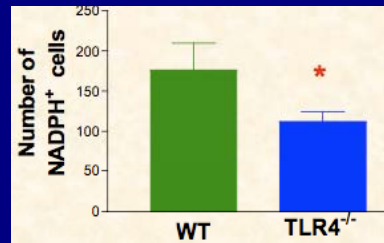
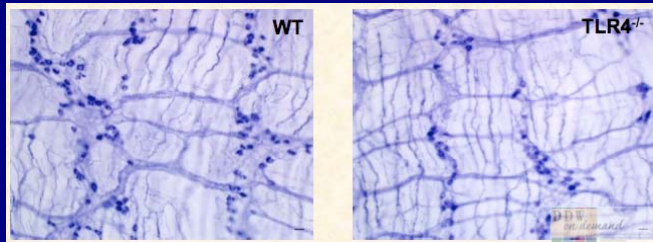
- TLR 4 mutants weighed less despite eating comparably
- Reduced stool frequency and daily stool weights

Intestinal transit delayed in TLR4 mutants

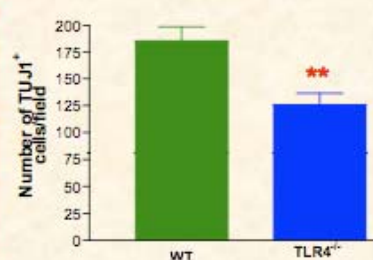
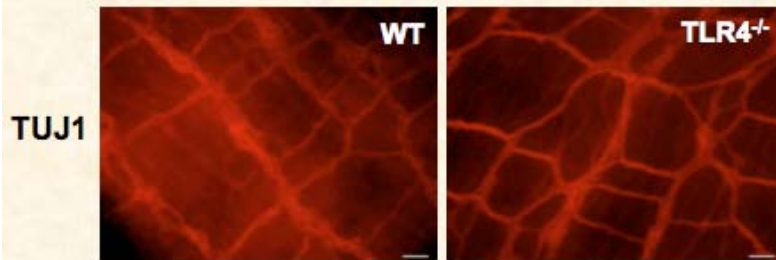


- Fasting mice fed fluorescent dye
- Sac'd 60 minutes after gavage
- Intestinal sections measured for fluorescence to quantify intestinal transit
- Colonic emptying measured as time to expel bead placed in mouse rectum

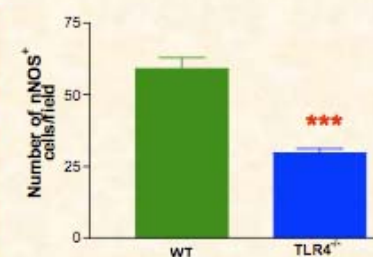
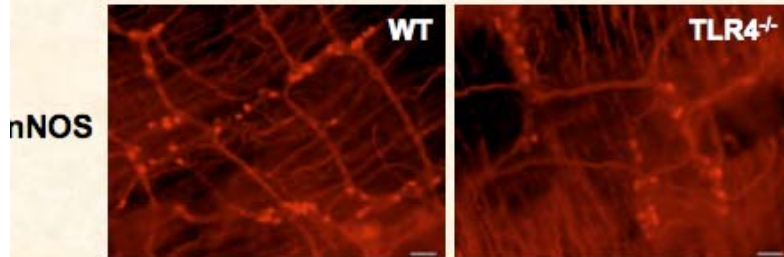
Reduced nitroergic neurons in myenteric plexus



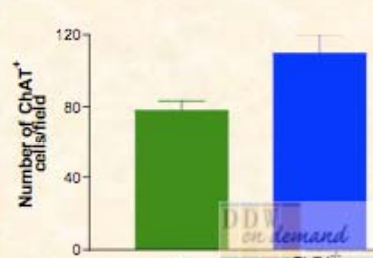
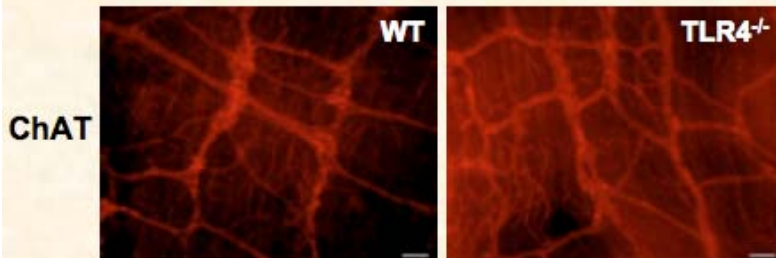
- Comparable staining and motility results in MyD88 KO mice



- Reduced nNOS neuron staining in GF mice with wildtype TLR signaling apparatus



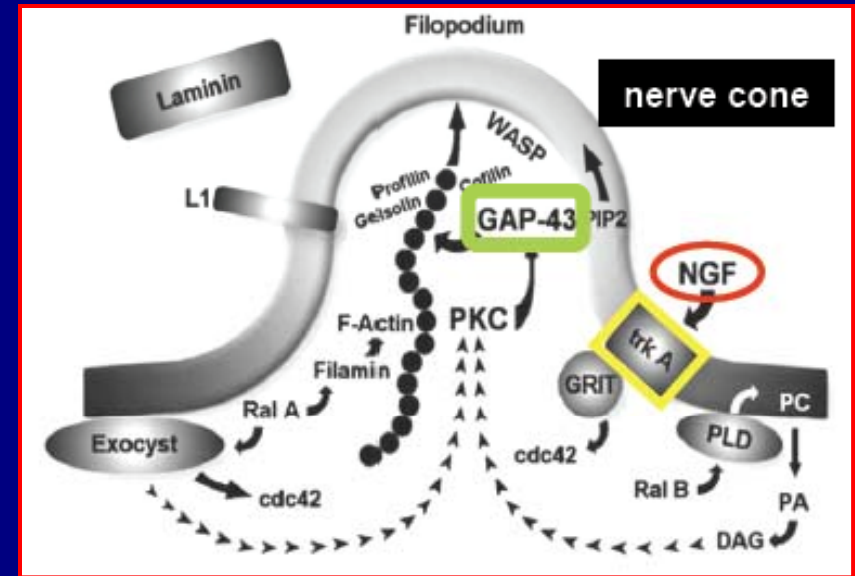
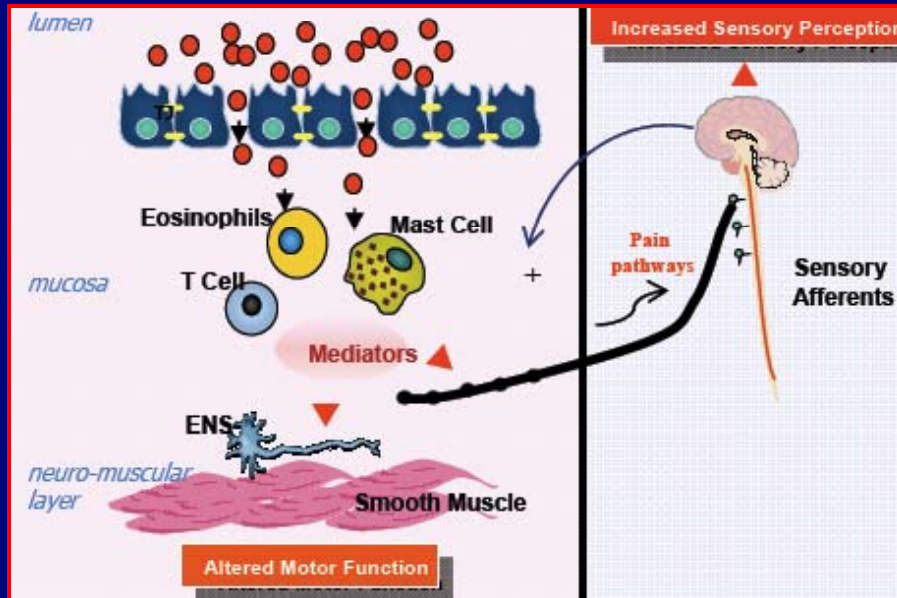
- Signal comes from gut flora



Bottom line (abstract #10)

- Lack of TLR4 signaling is associated with reduced colonic motility and number of nitrergic neurons
- Suggests bacterial-neuronal interaction in the developing ENS and in regulating GI motility
- Comment:
 - What is epithelial cell role?
 - Direct gut flora interaction with neuron?
 - Mechanistically intriguing. Clinical correlation less clear-cut. Differences will be much more subtle.

Nerve growth and plasticity in the colonic mucosa of patients with IBS (#475j)



- Mechanism of sensori-motor dysfunction of IBS remains unclear
- Hypothesis: abnormal intestinal milieu evokes long-term mucosal neuropathic changes that contribute to IBS
- NGF is candidate mediator of neuroplasticity; increased in inflammation, nerve overgrowth and visceral pain
- Growth-associated protein (GAP)-43 is activated by NGF, and increases axon sprouting

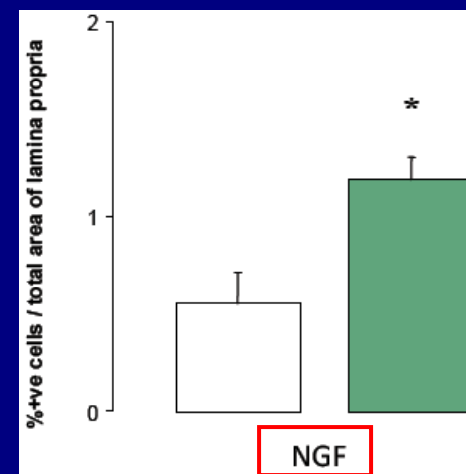
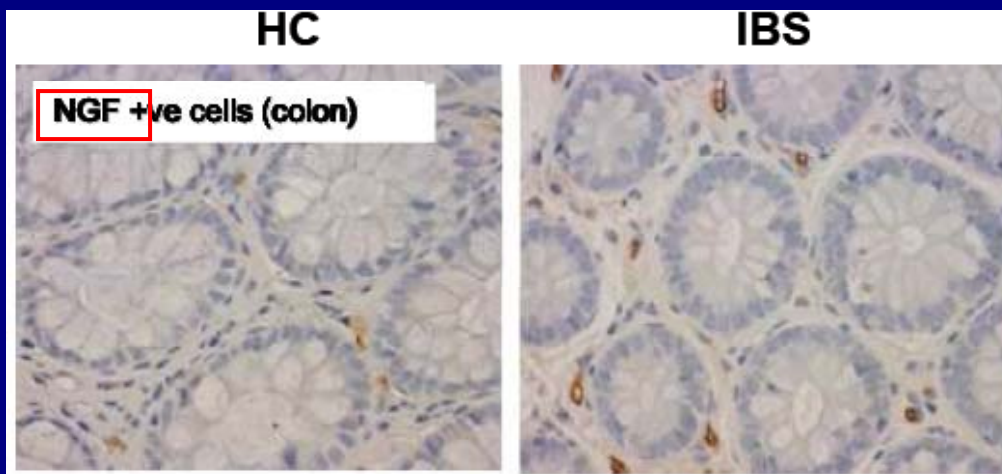
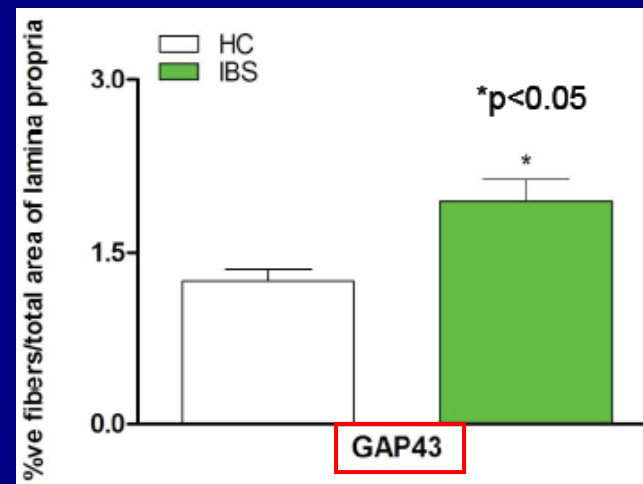
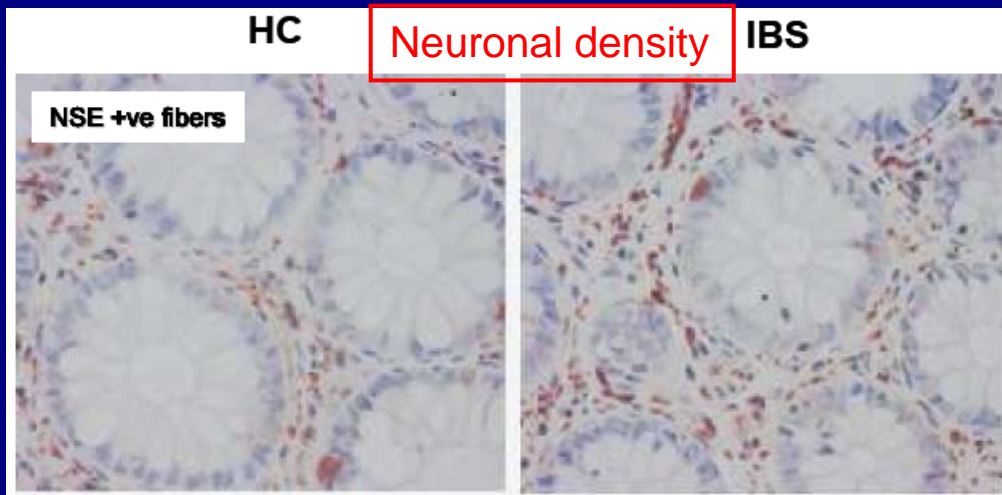
Question and approach

- What is the role of NGF and GAP-43 and neuroplastic changes in the intestines of IBS patients?
 - Neuronal density and sprouting in IBS colonic mucosa
 - Expression of NGF and GAP-43
 - Effect of these harvested mediators on human neuronal cell lines

Methods

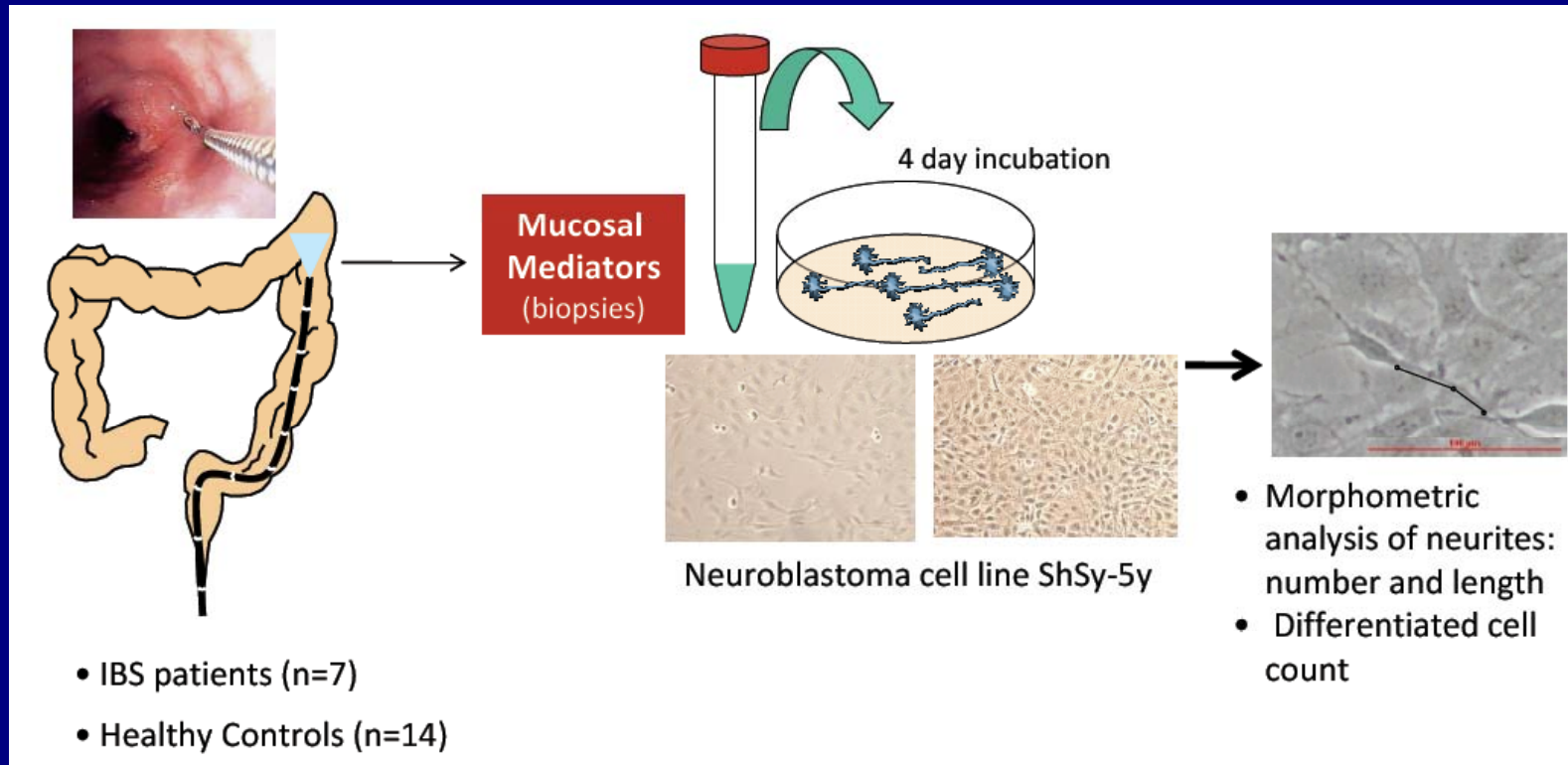
- 42 Rome III IBS patients (Diarrhea-12, Constip-18, mixed-12)
- 21 healthy controls
- Bx obtained from descending colon

Increased neuronal density and sprouting in IBS colonic mucosa

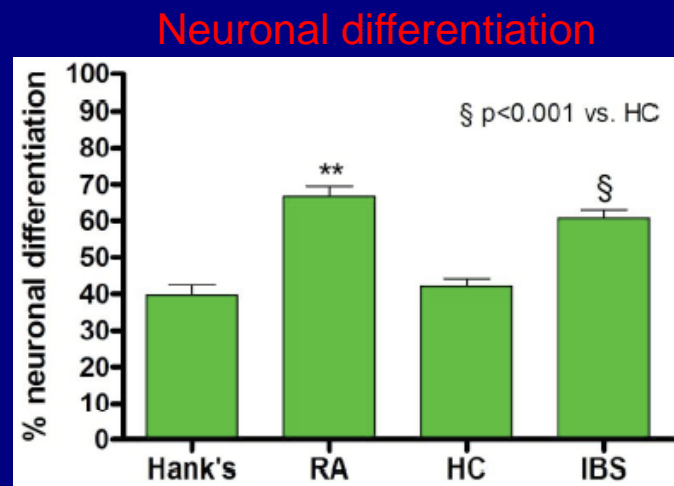
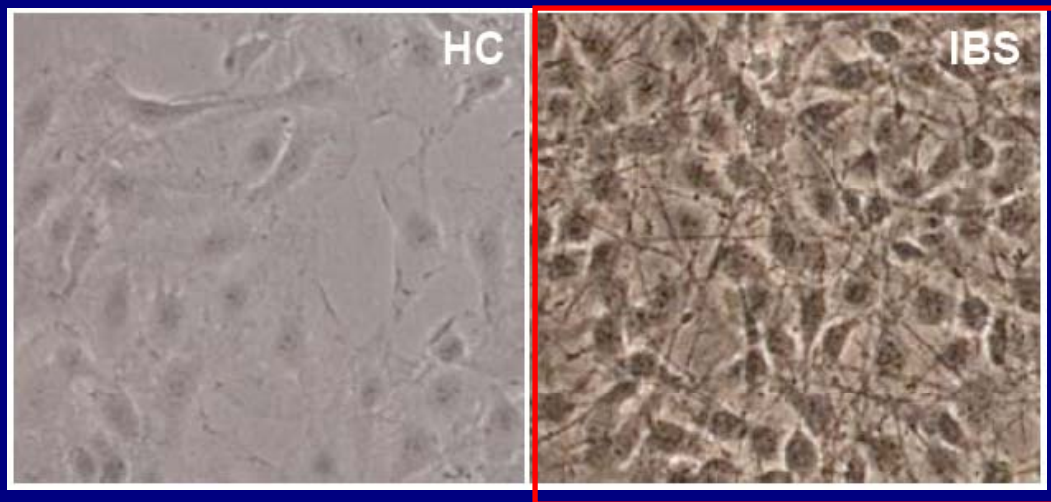
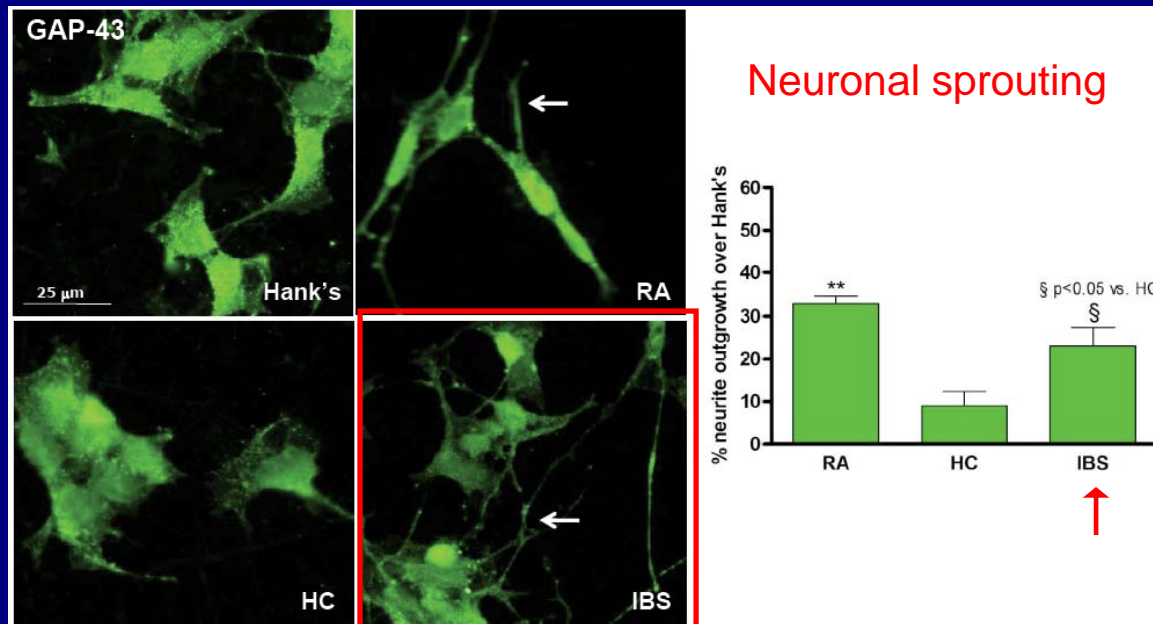


- Mucosal mast cells were main source of NGF

Effect of colonic mucosal mediators on neuronal cell differentiation and sprouting



Mediators from IBS colonic mucosa promote sprouting and differentiation of neurons



Authors' conclusions (abst #475j)

- Colonic mucosa from IBS patients show:
 - Increased nerve fiber density and GAP-43 related sprouting
 - Increased NGF (and TrkA)
- Transfer of mucosal mediators from IBS pts promote neuronal sprouting and differentiation
- Increased neuronal plasticity in mucosa of IBS pts. Mechanism for sensori-motor dysfunction?

Comment

- Greater differences between D-IBS vs C-IBS mucosa?
- PI-IBS samples?

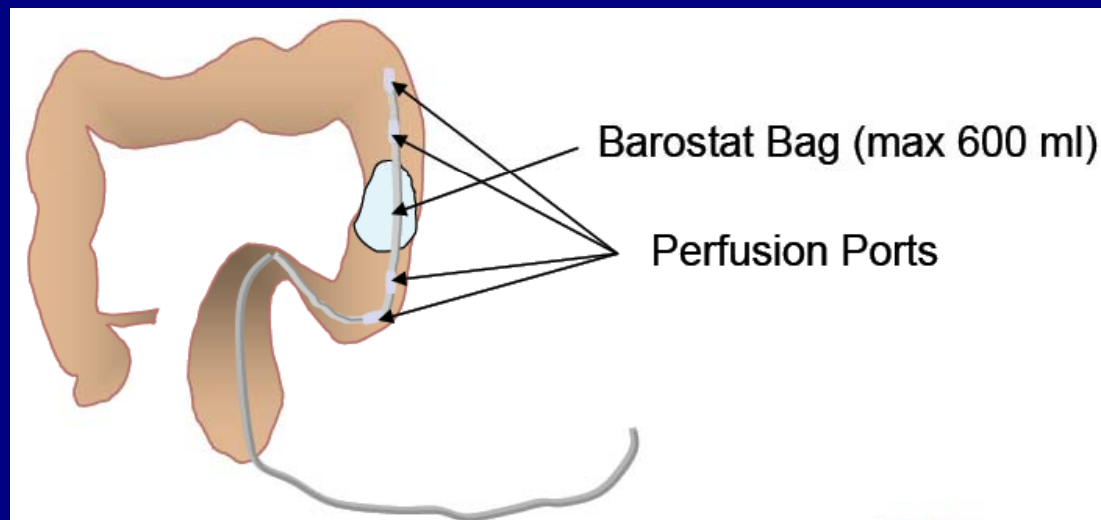
Motility response to colonic distention is increased in PI-IBS (Abstr #670)

- PI-IBS features:
 - Elevated enterochromaffin cells in rectal mucosa
 - Increased score on Hospital Anxiety and Depression Scale (compared to healthy and PI controls)
 - Similar score compared to non-PI IBS
- Question: Is PI-IBS associated with abnormalities in
 - Phasic contractions of colon
 - Smooth muscle tone
 - Pain sensitivity
 - Bowel symptoms
 - Psychological symptomsCompared to HC and non-PI IBS patients?

Methods

- 218 IBS Rome II pts (22 with PI-IBS); 43 healthy controls
- Questionnaires
 - IBS symptom severity scale
 - IBS QOL
 - Brief symptom inventory-18
- PI-IBS = Onset following acute illness (≥ 2 of Fever, N,V, +ve stool culture) without previous IBS symptoms

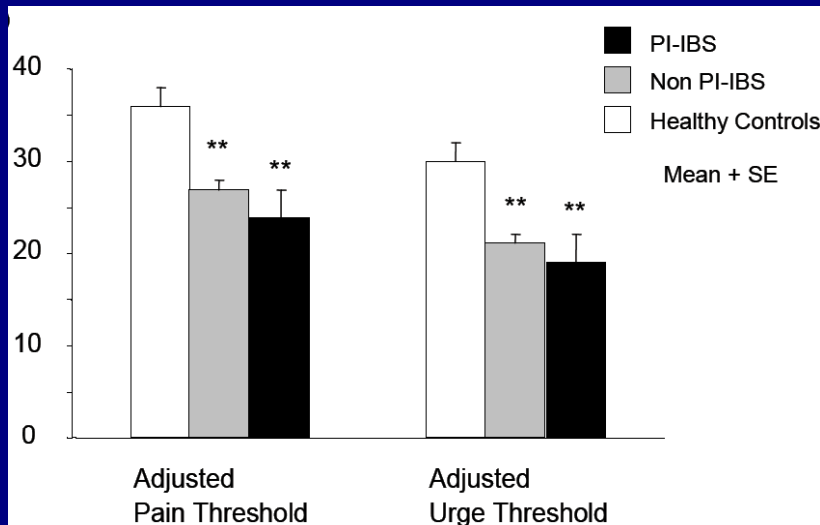
Measuring colonic motility and perception



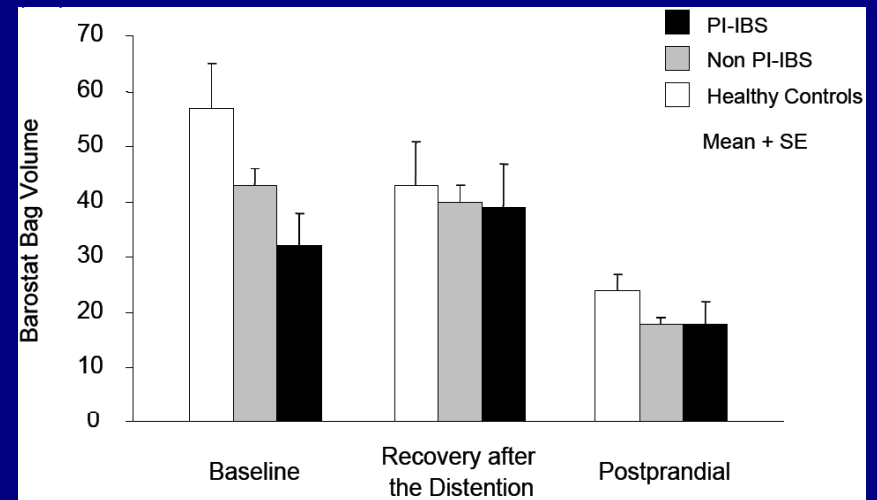
Questionnaire results

- PI-IBS and Non-PI IBS scored similarly in
 - IBS symptom severity
 - IBS QOL
 - Psychological features (Brief symptom index-18):
 - Somatization
 - Anxiety
 - Depression

No difference in colonic pain sensitivities between PI-IBS and non-PI IBS

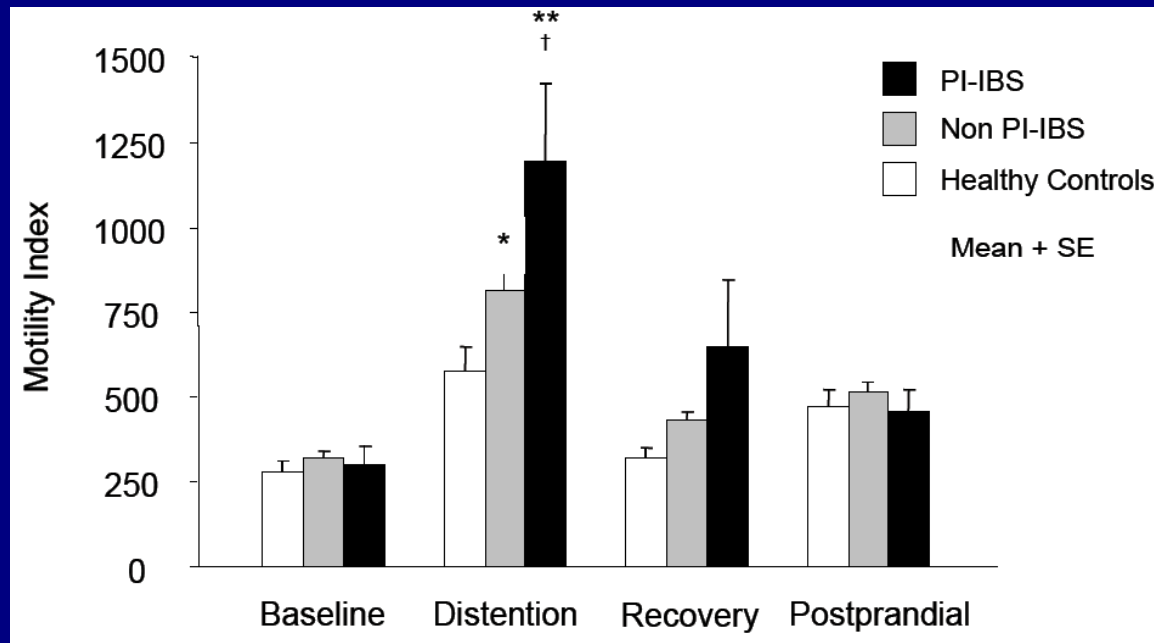


Colonic pain and urge perception to Intraluminal distention



Smooth muscle tone in descending colon

Colonic phasic motility response to distention is greater in PI-IBS than non-PI IBS



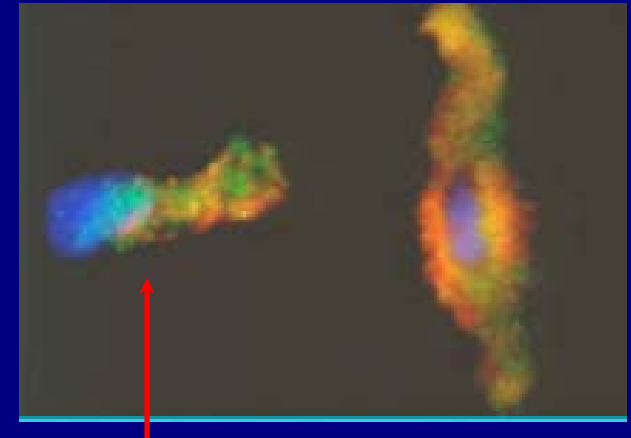
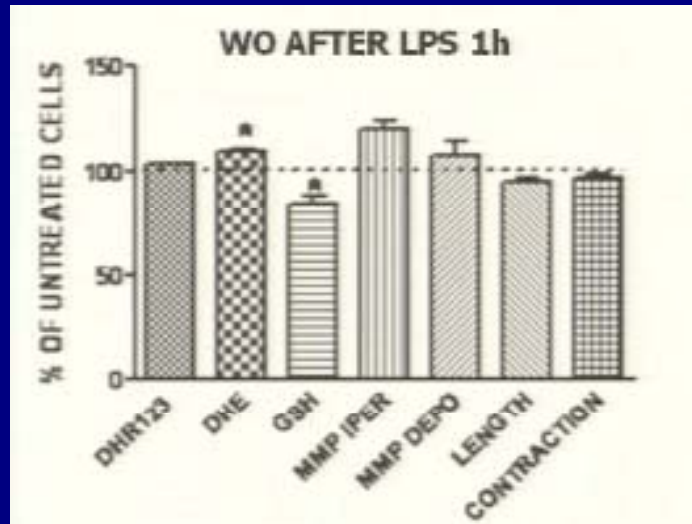
Conclusions (Abstr #670)

- Patients with PI-IBS have greater colonic hypercontractility than non-PI IBS
- No difference in colonic pain sensitivity and psychological tendencies between PI-IBS and non-PI IBS patients

Comment

- PI controls (No IBS after AGE) not assessed
- No colonic biopsies of mucosa to assess for inflammation, mast cell changes, etc.

Long term consequences induced by LPS exposure on human colonic smooth muscle: A possible mechanism contributing to PI-IBS (Abs#572)



Shortened HCSM cell

Recovery after 1hr exposure to LPS

- Duration and severity of acute GE is major risk for PI-IBS
- Human colonic smooth muscle (HCSM) cells can be maintained *in vitro* for 72 hours after isolation
- Alterations induced by 1 hr LPS exposure are reversible
- Question: does longer exposure to LPS cause persistent human colonic SM alterations → PI-FBD ?

Methods



Human colon circular
SM tissue



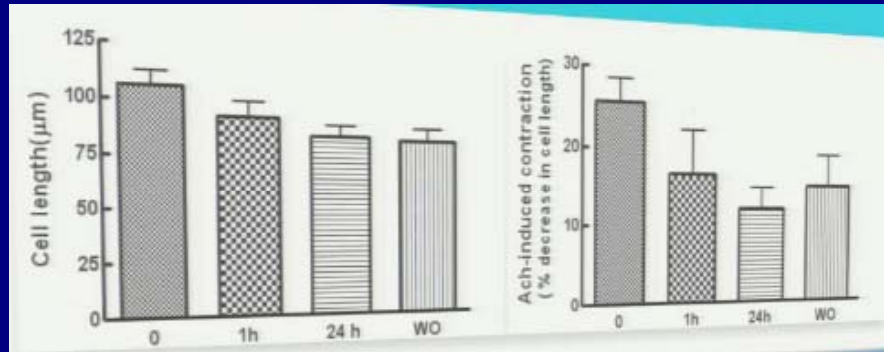
24 hr collagenase
and filtration



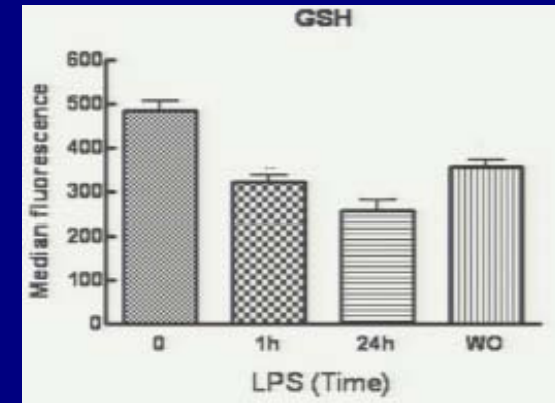
Primary culture
Survives 72 hrs

- HCSM in LPS 1 ug/ml for 24 hrs → washout for 24 hrs (LPS-free)
- LPS from pathogenic *E. coli* strain

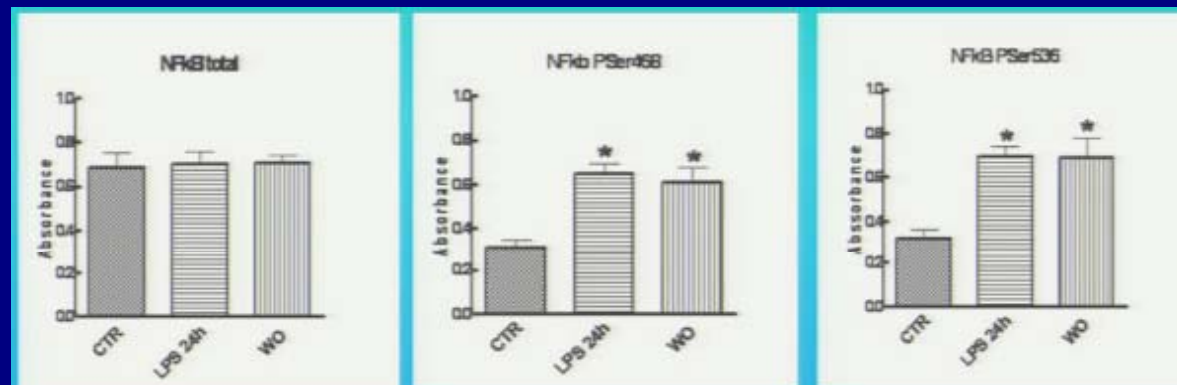
HCSM features not reversed after 24 hr washout in LPS-free media



HCSM cell shortening and contractile response not restored after washout

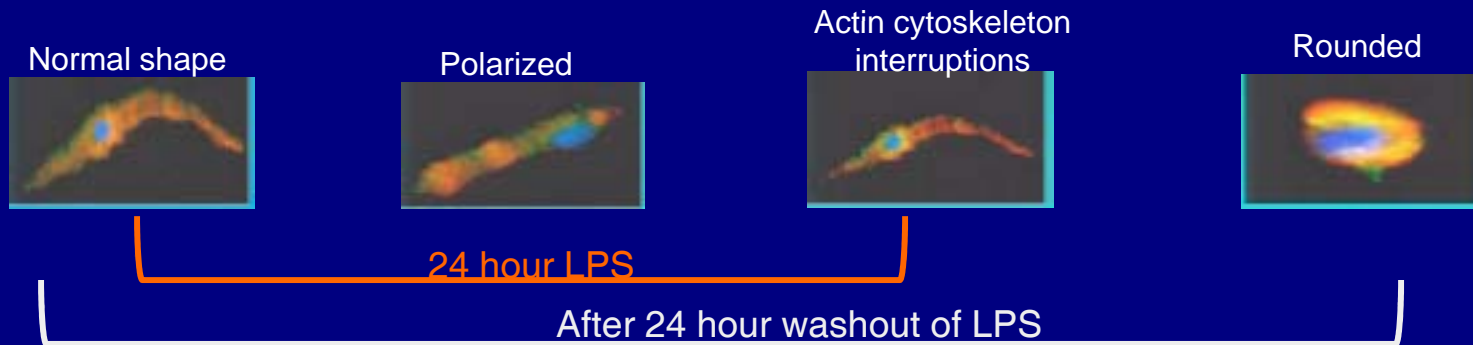


GSH content decrease and hyper-polarization of mitochondria membrane persisted after WO



Phosphorylated NF κ B p65 persisted after WO

Conclusions and comment (#572)



- Authors' Bottom Line:
 - Duration of exposure to bacterial Ag is critical for induction of persistent intestinal SM alterations
 - These alterations may contribute to PI motor disorders
 - New therapeutic target for PI-IBS?
- Comment:
 - Are these concentrations of LPS physiologic in acute GE?
 - Observations in an “isolated system” (no neuronal, immune or epithelial cells)

Intestinal methane production in obese humans is associated with higher BMI (Abstr #W1367)

- Gut microbiota contribute to obesity
- Methanogens associated with higher body weight in animals
 - Increased energy harvest?
 - Slowed intestinal transit?
 - Methane production by gut flora associated with constipation

Methods

- 58 subjects (74% female) recruited prospectively from wt loss clinic. BMI ≥ 30 .
- Symptom questionnaire (with VAS), medication hx
- Breath sample tested for methane. Positive if ≥ 3 ppm
- Bivariate and multivariate analyses performed



Abstract #W1367

	Methane neg	Methane pos	P value
BMI	38.5 \pm 0.8	45.2 \pm 2.3	0.001
Constip (VAS)	9.5 \pm 2.4	21.3 \pm 6.4	0.043

- 12/58 (21%) methane positive
- Bi-variate analysis: constipation and anti-depressant use were confounding variables
- Controlling for both: methane still predictor of elevated BMI
- **Comment:**
Further example of gut bacteria influence on metab and obesity. Animal gut flora manipulation experiments may clarify mechanism.

Fecal bacteriotherapy for recurrent *C. difficile* infection (abstract #29)



- Prospective study. 37 patients from 3 Finnish hospitals.
- Failed ≥ 3 courses metronidazole or vancomycin
- 30 of 37 received stool transplant from relative
- Question: Does it work?

Methods

- Donors screened: *C.diff*, O+P, enteric pathogens, HAV, HBV, HCV, HIV, and *T. pallidum* infection
- Fecal transplant procedure:
 - PEG bowel prep
 - Abx held for ≥ 36 hours before transplant
 - 3 hours before-- 20-30 ml donor stool manually homogenized with 100-200 ml sterile water
 - Stool suspension delivered via colonoscope biopsy channel into cecum

Success!

Hospital type	Number of patients with CDI	Age (range, mean)	RNA ribotype 027 positive	Number of fecal infusions	Follow up months	Cured [±] n/total (%)
Central	19	37-87 (71)	3/19 (5 NA)	1-2	3 and 12	18/19 (95)
University	10	24-81 (60)	4/10	1	2 and 12	10/10 (100)
Municipal	8	60-90 (79)	4/8 (3 NA)	1	2 and 12	6/8 (75)

*No CDI relapses during 12 months.

Cured 92 %

- Phone f/u at 3 months. Med record check at 1 year.
- Diarrhea resolved in most patients w/in 2-3 days
- Treatment failures were older inpatients
- Two relapsed after new Abx course. Cured again with fecal re-infusion

Authors' conclusion

Fecal transplantation is safe and effective for recurrent *C.difficile* infection

Comment

- *C. diff* toxin status not checked post-infusion
- Antibody levels against *C. diff* in donors not measured
- Less expensive than infusion of monoclonal anti-toxin antibody?
- http://www.nytimes.com/2010/07/13/science/13micro.html?_r=1&emc=eta1

FBD pathophys: Take home messages

- Role of gut flora in FBD
 - Disruption of early gut population led to visceral hypersensitivity
 - Gut microbiome influences CNS neurotransmission
 - Absence of microbe-host signaling (TLR pathway) leads to altered GI motility and ENS development
- Mechanistic insights
 - Increased neuronal plasticity in gut mucosa of IBS patients
 - PI-IBS patients have greater colonic hypercontractility than non-PI IBS pts. No difference in psychological “tendencies”
 - Prolonged alterations in human colonic smooth muscle cells after protracted bacterial Ag exposure
- Intestinal methane production predicts higher BMI in obese patients
- Fecal bacteriotherapy is effective for recurrent *C. difficile* infection