De ‘microbiota-gut-brain axis’
*Tussen hype en revolutie?*

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- Translational Research Center for Gastrointestinal Disorders (TARGID)
- KU Leuven
Introduction
anatomy & physiology of the ‘brain-gut axis’
Gastrointestinal Tract
Gastrointestinal Tract
an extremely complex organ system

• **surface** ~ 300 m²
  o absorption

• **nervous organ**
  o 100 million nerve cells
    • “intrinsic”
    • “extrinsic”
  o most complex interaction with the brain

• **hormonal organ**
  o largest hormone production (> 30 hormones)

• **immune organ**
  o 60-70% of our immune cells

• $10^{14}$ **bacteria**
Enteric Nervous System (ENS)
Emotional Brain

- **Brainstem**
  - PAG, periaqueductal gray
  - LC, locus coeruleus

- **Subcortical**
  - NA, nucleus accumbens
  - AMG, amygdala

- **Cortical**
  - ACC, anterior cingulate cortex
  - OFC, orbitofrontal cortex
Gut-Brain Axis

**HUMORAL**
GUT-BRAIN
- gastrointestinal hormones
- inflammatory mediators
- BRAIN-GUT
- Hypothalamo-Pituitary-Adrenal Axis

**NEURAL**
- Autonomic Nervous System
Vagus nerve
Insula
Linking sensory & emotional information
Hypothalamo-Pituitary-Adrenal (HPA) axis
1. The link between psychological & gastrointestinal symptoms
‘stress-related’ gastrointestinal symptoms normality to functional gastrointestinal disorders

• normality
  o gastrointestinal symptoms during stress
    • stomach problems
    • diarrhea
  o “butterflies in the stomach”

• “functional gastrointestinal disorders”
  o irritable bowel syndrome, functional dyspepsia,…
  o symptom-based diagnosis, no organic cause
  o associated with stress, fear/anxiety, depression,…
irritable bowel syndrome (IBS)
Rome IV definition

C1. Diagnostic Criteria\textsuperscript{a} for Irritable Bowel Syndrome

Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria:

1. Related to defecation
2. Associated with a change in frequency of stool
3. Associated with a change in form (appearance) of stool

\textsuperscript{a}Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.
irritable bowel syndrome
world prevalence
functional gastrointestinal disorders
biopsychosocial model

Early Life
- Genetics
- Culture
- Environment
  - Trauma
  - Infection
  - Parental behaviors

Psychosocial Factors
- Life stress
- Personality traits
- Psychologic state
- Coping/cognitions
- Social support

Brain
- CNS

Gut
- ENS

Physiology
- Motility
- Sensation
- Immune dysfunction/inflammation
- Altered microflora
- Food/diet

FGID
Presentation
- Symptoms
- Severity
- Behaviors

Outcome
- Health Care Use
- Daily function
- Quality of life
- Health Care Costs
Functional Gastrointestinal Disorders

disorders of gut-brain interactions

symptom reporting

visceral perception

afferent nerves

mechanoreceptors

mechanical GI stimulation

GI motor (dys)function

GI immune & barrier function

Gut microbiota

CRF

HPA-axis

ANS

descending modulatory pathways

dorsal horn

spinal (orthosympathic)

vagal (parasympathic)

sensorimotor network

affective cognitive networks

autonomic network

Brain modulatory pathways

Brain modulatory pathways

CRF

CRF

CRF
Psychological impact on GI symptoms overview

- Symptom reporting
- Visceral perception
- Affective cognitive networks
  - Sensorimotor network
- Brain modulatory pathways
- Vagal (parasympathetic)
- Dorsal horn
- Spinal (orthosympathetic)
- Afferent nerves
- Mechanoreceptors
- GI immune & barrier function
  - Gut microbiota
- CRF
- Autonomic network
- HPA-axis
  - ANS
- GI motor (dys)function
  - CRF
HPA-axis & gastrointestinal responses to CRH injection in IBS

Experiment 1

- CRH 2 µg/kg i.v.
- Blood sample
- Rectal motor response / ECG
- Parameters:
  - HPA axis: ACTH & cortisol
  - Colonic motor responses: number of PVEs/5 min
  - Autonomic function: HRV/5 min

Experiment 2

- Brain activity during rectal distension correlated with the ACTH-AUC
- Visual cue:
  - Anticipation: 9-18 s
  - Distention: 18 s
  - Rating and rest: 24 s

Kano, ..., Van Oudenhove, ..., Fukudo, Sci Rep 2017
Increased ACTH response to CRH injection in IBS
Increased colonic motility response to CRH injection in male IBS patients

Kano, ..., Van Oudenhove, ..., Fukudo, Sci Rep 2017
Psychological impact on GI symptoms overview

- Symptom reporting
- Visceral perception
- Afferent nerves
- Mechanoreceptors
- Mechanical GI stimulation
- GI motor (dys)function
- Vagal (parasympathetic)
- Spinal (orthosympathetic)
- Descending modulatory pathways
- Sensorimotor network
- Affective cognitive networks
- Brain modulatory pathways

- GI immune & barrier function
- Gut microbiota
- CRF
- HPA-axis
- ANS
- CRF
- CRF
Psychosocial stress increases small intestinal permeability in healthy subjects

**Vanuytsel et al, Gut 2013**

**Salivary Cortisol (ng/ml)**

***

**0**

**5**

**10**

**15**

**20**

**Basal**

**Speech**

**Shock**

Psychosocial stress increases small intestinal permeability in healthy subjects

**Vanuytsel et al, Gut 2013**

**STAI score**

***

**0.00**

**0.02**

**0.04**

**0.06**

**Basal**

**Speech**

**Shock**

**LMR**

**mixed models + Bonferroni:**

**P<0.01 vs. basal**

**P<0.001 vs. basal**

**P<0.05 vs. speech**

**Salivary Cortisol (ng/ml)**

**Basal**

**Speech**

**Shock**

**p=0.0028**

**p=0.22**

**p<0.0001**

**cortisol**

**stressor**

**LMR**
Psychological impact on GI symptoms overview

- Symptom reporting
- Visceral perception
- Sensorimotor network
- Affective cognitive networks
- Brain modulatory pathways

- Dorsal horn
- Descending modulatory pathways
- Autonomic network

- Afferent nerves
- Mechanoreceptors
- Mechanical GI stimulation
- GI motor (dys)function
- GI immune & barrier function
- Gut microbiota

- Spinal (orthosympathic)

- CRF
- HPA-axis
- ANS
gastrointestinal sensitivity testing

Schema for Evaluation of Enteric Sensation and Reflexes in the Functional GI Disorders

(1) Apply test stimuli
- Physiological e.g., oral or intraluminal nutrients
- Supraphysiological e.g., gut distension

(2) Measure responses
- Conscious perception
- Afferent signals in brain, spinal cord
- Autonomic responses
- Reflex gut motor activity

(3) Account for central and local modulation of responses
gastrointestinal hypersensitivity
Emotional modulation of visceral pain paradigm

"Emotion induction period"  "Emotional pain modulation period"

“time 0”  “time 1”  “time 2”

Emotion induction
- Validated classical music
- Velten mood induction statements
- IAPS pictures

VAS  SAM  ES  ES  ES  ES  ES  ES  ES  ES

"time 0"  "time 1"  "time 2"

start emotion induction  stop emotion induction

Weltens, Schaub, Aziz, Tack, Van Oudenhove* & Coen*, submitted
Emotional modulation of visceral pain
sad emotion induction
Emotional modulation of visceral pain effect on esophageal pain ratings

Weltens, Schaub, Aziz, Tack, Van Oudenhove* & Coen*, submitted

![Graph showing pain intensity and emotion conditions]

- Negative
  - Pain intensity (0 - 100)
  - % Δ in pain intensity
  - β = -0.064; p = 0.0026

- Neutral
  - Pain intensity (0 - 100)
  - % Δ in pain intensity
  - β = -0.48; p = 0.0026

- Positive
  - Pain intensity (0 - 100)
  - % Δ in pain intensity
  - β = -0.72; p = 0.0001

![Graph showing stimulus number and pain intensity]

Stimulus number

Weltens, Schaub, Aziz, Tack, Van Oudenhove* & Coen*, submitted
Emotion, cognition & pain

Bushnell et al, Nature Reviews Neuroscience 2013
Emotion, cognition & pain

- PAG: periaqueductal gray
- PB: parabrachial nucleus
- AMY: amygdala
- BG: basal ganglia
- S1, S2: somatosensory cortex
- ACC: anterior cingulate cortex
- PFC: prefrontal cortex

*Bushnell et al, Nature Reviews Neuroscience 2013*
Emotion, cognition & pain

- PAG: periaqueductal gray
- PB: parabrachial nucleus
- AMY: amygdala
- BG: basal ganglia
- S1, S2: somatosensory cortex
- ACC: anterior cingulate cortex
- PFC: prefrontal cortex
- SPL: superior parietal lobule

Bushnell et al, Nature Reviews Neuroscience 2013
descending pain modulatory pathways

[Image of a brain diagram showing descending visceral pain pathways with labels for Thalamus, PAG, Locus coeruleus, Caudal raphe nucleus, Amygdala, Noradrenergic, Serotonergic, Opioidergic, ACC, Rostral ventral medulla, Colon, and Spinal cord.]
3. impact of the gut microbiota on psychological & brain function
Microbiota-Gut-Brain Axis
The hype of the day?

Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour

John F. Cryan¹,² and Timothy G. Dinan¹,³

Gut Microbes and the Brain: Paradigm Shift in Neuroscience

Emeran A. Mayer,¹ Rob Knight,² Sarkis K. Mazmanian,³ John F. Cryan,⁴ and Kirsten Tillisch⁵

REVIEW

Psychobiotics: A Novel Class of Psychotropic

Timothy G. Dinan, Catherine Stanton, and John F. Cryan

What’s bugging your teen?—The microbiota and adolescent mental health

Karen-Anne McVey Neufeld², Pauline Luczynski³, Clara Seira Oriach⁴,⁵, Timothy G. Dinan³,⁴, John F. Cryan³,⁵,
Gut microbiota

- **collection of micro-organisms** in our gastrointestinal tract
- **bacteria**
  - $10^{14}$ in the colon (~ 10 x human cells in the entire body)
  - > 1000 species (but ~ 200 in each individual)
  - 3 x $10^6$ genes (~ 100 x human genes)
  - 1 – 2 kg weight
- **variability** between individuals
  - host genetics
  - birth mode (caesarian section versus vaginal delivery)
  - breastfed versus bottle-fed
  - antibiotics
  - diet, diet, diet!
  - stress
  - age
## Gut microbiota

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>64%</td>
</tr>
<tr>
<td>- Lactobacillus</td>
<td></td>
</tr>
<tr>
<td>- Streptococcus</td>
<td></td>
</tr>
<tr>
<td>- Staphylococcus</td>
<td></td>
</tr>
<tr>
<td>- Enterococcus</td>
<td></td>
</tr>
<tr>
<td>- Faecalibacterium prausnitzii</td>
<td></td>
</tr>
<tr>
<td>- Clostriádia (C. difficile)</td>
<td></td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>23%</td>
</tr>
<tr>
<td>- Bacteroides</td>
<td></td>
</tr>
<tr>
<td>- Prevotella</td>
<td></td>
</tr>
<tr>
<td>- Alistipe;</td>
<td></td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>8%</td>
</tr>
<tr>
<td>- Enterobacteriaceae</td>
<td></td>
</tr>
<tr>
<td>(Salmonella, E. coli, Klebsiella, Shigella)</td>
<td></td>
</tr>
<tr>
<td>- Campylobacter (C. jejuni)</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>3%</td>
</tr>
<tr>
<td>- Bifidobacteria</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>98%</td>
</tr>
</tbody>
</table>
Gut microbiota
Microbiota-Gut-Brain Axis

What?
Microbiota-gut-brain axis
Mechanisms
Microbiota-gut-brain axis
How to study?
Microbiota-gut-brain axis definitions

• **probiotics**
  - “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”

• **prebiotics**
  - “a substrate that is selectively utilized by host microorganisms conferring a health benefit”

• **“psychobiotics”**
  - either of the above conferring a health benefit *in patients with psychiatric illness*
Microbiota-gut-brain axis
fecal microbiota transplantation
How to test anxious behaviour in mice? elevated plus maze test
How to test anxious behaviour in mice?

open field test
How to test depressive behaviour in mice?
forced swim test
Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Javier A. Bravo, Paul Forsythe, Marianne V. Chew, Emily Escaravage, Hélène M. Savignac, Timothy G. Dinan, John Bienenstock, and John F. Cryan

Proceedings of the National Academy of Sciences USA 2011

**stress-induced hyperthermia**

**elevated plus maze**

**forced swim test**

**response to forced swim test**

**Corticosterone (ng/mL)**

Bravo, Forsythe et al, Proceedings of the National Academy of Sciences USA 2011
L. Rhamnosus & central GABA receptors

Bravo, Forsythe et al., Proceedings of the National Academy of Sciences USA 2011
Obese-type Gut Microbiota Induce Neurobehavioral Changes in the Absence of Obesity

A

![Graph showing body weight changes over experiment duration](image)

- **Body Weight (grams)**
  - **ABX**
  - **Microbiome transplant**
  - **Behavioral tests**

B

![Graph showing elevated plus maze time in open arms](image)

- **Elevated Plus Maze Time in open arms (% total test time)**
  - CD
  - HFD

C

![Graphs showing open field test and overall locomotor activity](image)

- **Open Field Test**
  - Time in inner zone (% total time)
  - Mean speed (meters/sec)
  - Total distance (meters)

Bruce-Keller et al, Biol Psychiatry 2015
How to measure emotional responses in humans? Affect labeling/matching task

**Congruent trial**

- Fixation (1000ms)
- Target (500ms)
- Probe (100ms)
- Response “Did you see ‘:’ or ‘:’?” (1000ms)

- Neutral face
- Angry face

**Incongruent trial**

- Fixation (1000ms)
- Target (500ms)
- Probe (100ms)
- Response “Did you see ‘:’ or ‘:’?” (1000ms)

- Neutral face
- Angry face
Prebiotic B-GOS alters cortisol awakening response and emotional bias in health

no differences on
• 2 other emotion tasks
  • facial expression recognition
  • emotional categorization & memory
• self-report questionnaires
  • anxiety
  • stress

Schmidt et al, Psychopharmacology 2015
Probiotic intake alters resting state brain function in healthy humans

Tillisch, ..., Mayer, Gastroenterology 2013
How to measure emotional brain response in humans?
Affect labeling/matching task
Probiotic intake alters brain responses to emotional attention task in healthy humans

Tillisch,..., Mayer, Gastroenterology 2013
How to measure emotional brain response in humans? Backward masking paradigm
Probiotic treatment decreases depressive symptoms & emotional brain responses in IBS

Pinto Sanchez et al, Gastroenterology 2017
Altered microbiota composition in major depressive disorder

Microbiota from depressed patients induce depressive & anxious behaviour in germ-free mice

forced swim test


tail suspension test

overall locomotor activity

open field test
Microbiota from anxious IBS patients induce anxious behaviour in germ-free mice

De Palma et al, Sci Transl Med 2017
Take home messages

- There is intimate **bidirectional communication** between the **gastrointestinal tract** and the **brain**

- This constitutes the **biological basis** of the **link** between **psychological** and **gastrointestinal symptoms**

- The **gut microbiota** have recently been identified as a key **new player** in this **communication**

- Therefore, the **microbiota** could have a strong impact on our **psychological and brain function**, but translation from animals to humans has only just started