MICROBES, BRAIN AND BEHAVIOUR

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Infection studies

Subclinical infection with Campylobacter jejuni results in anxiety behaviour (Lyte et al, 1998)

Walkerton study.. contamination of municipal water by Escherichia coli 0157:H7 and Campylobacter species
Minocycline and psychiatric symptoms

*Minocycline* has broad *spectrum activity* against both gram positive and gram negative organisms.

Novel therapeutic targets in depression: *minocycline* as a candidate treatment.

Behav Brain Res 2012

Successful use of add-on *minocycline* for treatment of persistent negative symptoms in schizophrenia.

J Neuropsychiatry Clin Neurosci. 2013
Faecal Microbiota Transplantation

? Neurodegenerative disorders

? Neurodevelopmental disorders

T.J. Borody and A. Khoruts Nat Rev Gastroenterol Hepatol. 2011
Germ-free in early life – effects on the social brain?

Microbiome-gut-brain axis

Germ-free mouse

Altered gut-brain communication

↓ memory

↑ brain serotonin

Altered social behaviours??

CRH

Pituitary

ACTH

Hypothalamus

Mood Cognition Emotion

Adrenal

Cortisol

Vagus Nerve

Enteric muscle

Epithelium

Enteric microbiota

Short-chain fatty acids

Neurotransmitters

Cytokines

Immune cells

5-Hydroxytryptophan

Serotonin

↑↑ brain serotonin

Altered social behaviours??
BDNF is a neurotrophin supporting neuronal survival/growth

Decreased BDNF in germ free compared to control animals

Altered stress response

Grenham et al., 2011
Altered CNS Serotonergic Function

- Elevated concentrations of 5-HT in hippocampus of germ free animals

- Increased 5-HT turnover in brainstem

Clarke et al, 2012
Three-chamber sociability test

1. **Habituation**: exploration of 3 chambered box (10 mins).

2. **Sociability**: does the test mouse spend more time in the chamber containing the mouse or in the opposite empty chamber?

3. **Social novelty preference**: does the test mouse spend more time in the chamber containing the now familiar mouse or in the opposite chamber containing a new ‘strange’ mouse?

Desbonnet et al, Mol Psychiatry 2013
Germ free effects on sociability

![Sociability graph]

- Female
- Male

**Sociability**

- Empty
- Mouse

Time (s)

C

Conventional

Germ Free

Mouse Empty
Germ free effects on preference for social novelty

Preference for social novelty

![Graph showing preference for social novelty with asterisks indicating significance levels.](image)

- **Time (s)**
- **Preference for social novelty**

- **Familiar**
- **Novel**

- **Female**
- **Male**

- **Germ Free**
- **Conventional**

Novel Familiar

C F GFR C GFR

0 50 100 150 200 250 300 350
Microbiota: Which species is the most effective neurochemical producer?

400–500 species including:
- Bacteroides
- Eubacterium
- Peptostreptococcus
- Bifidobacterium
- Ruminococcus
- Bacillus
- Fusobacterium
- Clostridium
- Lactobacillus
- Enterococcus
- Enterobacter

**Anaerobes**

**Aerobes**

Jejunum: $10^{3-4}$

Terminal Ileum: $10^{7-9}$

Colon: $10^{10-12}$

Many bacteria remain unculturable…….?15%
What neurotransmitters can be produced by microbes?

- Norepinephrine: Escherichia, Bacillus, and Saccharomyces
- Serotonin: Streptococcus, Escherichia, and Enterococcus
- Dopamine: Bacillus and Serratia

Roschina, 2010
What neurotransmitters can be modulated by bacteria?

- *Lactobacillus acidophilus* strain modulates expression of cannabinoid receptors in the spinal cord

*Bif. Infantis* increases plasma tryptophan levels and thereby modulates 5HT

*Lactobacillus rhamnosus* alters central GABA receptor expression
Lactobacillus strain, GABA receptor expression and behaviour

Bravo et al, PNAS, 2011
Bravo et al, PNAS, 2011
Effects of Vagotomy

Bravo et al, PNAS, 2011
Can probiotic ingestion affect brain function in humans?

- 45 Healthy women
  - Age 18-50 (mean age 29 years)
  - No gastrointestinal symptoms
  - No chronic pain
  - No psychiatric illness
  - No probiotic or antibiotic use in the last month

- **Test product (n= 15)**
  - Commercially available fermented milk product (FMPP)
  - 125 grams twice/day for 4 weeks

- **Non-fermented dairy product (n= 12)**
  - 125 grams twice/day for 4 weeks

- **No treatment (n=14)**
Intake of FMPP was associated with decreased connectivity of an extensive brain network including somato- and viscerosensory regions in response to the task.

Across visits the network becomes:
- stronger with no treatment
- stays the same with control
- decreased in Test Product.

Network crossblock covariance 49%, P <.005

Tillisch, et al, Gastroenterology 2013
Here, we define a psychobiotic as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. As a class of probiotic, these bacteria are capable of producing and delivering neuroactive substances such as gamma-aminobutyric acid and serotonin, which act on the brain-gut axis. Preclinical evaluation in rodents suggests that certain psychobiotics possess antidepressant or anxiolytic activity. Effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. So far, psychobiotics have been most extensively studied in a liaison psychiatric setting in patients with irritable bowel syndrome, where positive benefits have been reported for a number of organisms including Bifidobacterium infantis. Evidence is emerging of benefits in alleviating symptoms of depression and in chronic fatigue syndrome. Such benefits may be related to the antiinflammatory actions of certain psychobiotics and a capacity to reduce hypothalamic-pituitary-adrenal axis activity. Results from large scale placebo-controlled studies are awaited.
Appropriate targets for psychobiotics

? Depression/Anxiety
*L. helveticus* together with *B. longum*
↓ psychological distress relative to placebo and
↓ urinary free cortisol output (*Messaoudi et al, 2011*)

? Chronic fatigue syndrome
↓ anxiety in those given *L. casei* relative to placebo (*Rao et al, 2009*)

*Irritable bowel syndrome* (*Whelan & Quigley, 2013*)
Major Gaps

- Paucity of human studies
- Which route of communication between gut microbes and brain is most important in man?
- Do patients with psychiatric illness have a distinct microbiota fingerprint?
- Do probiotics produce an anxiolytic/antidepressant effects in humans that have been reported in rodents?
Strategy for Identifying Psychobiotics

1. Establish a library of putative probiotics
2. Culture and obtain supernatants
3. Subject supernatants to GC/MS and identify probiotics producing neuroactive compounds
4. Examine supernatant action on neuronal cell lines
5. Determine viability on gastric transit
6. Examine probiotic in animal models
7. Human intervention studies
How do probiotics alter stress related behaviour?