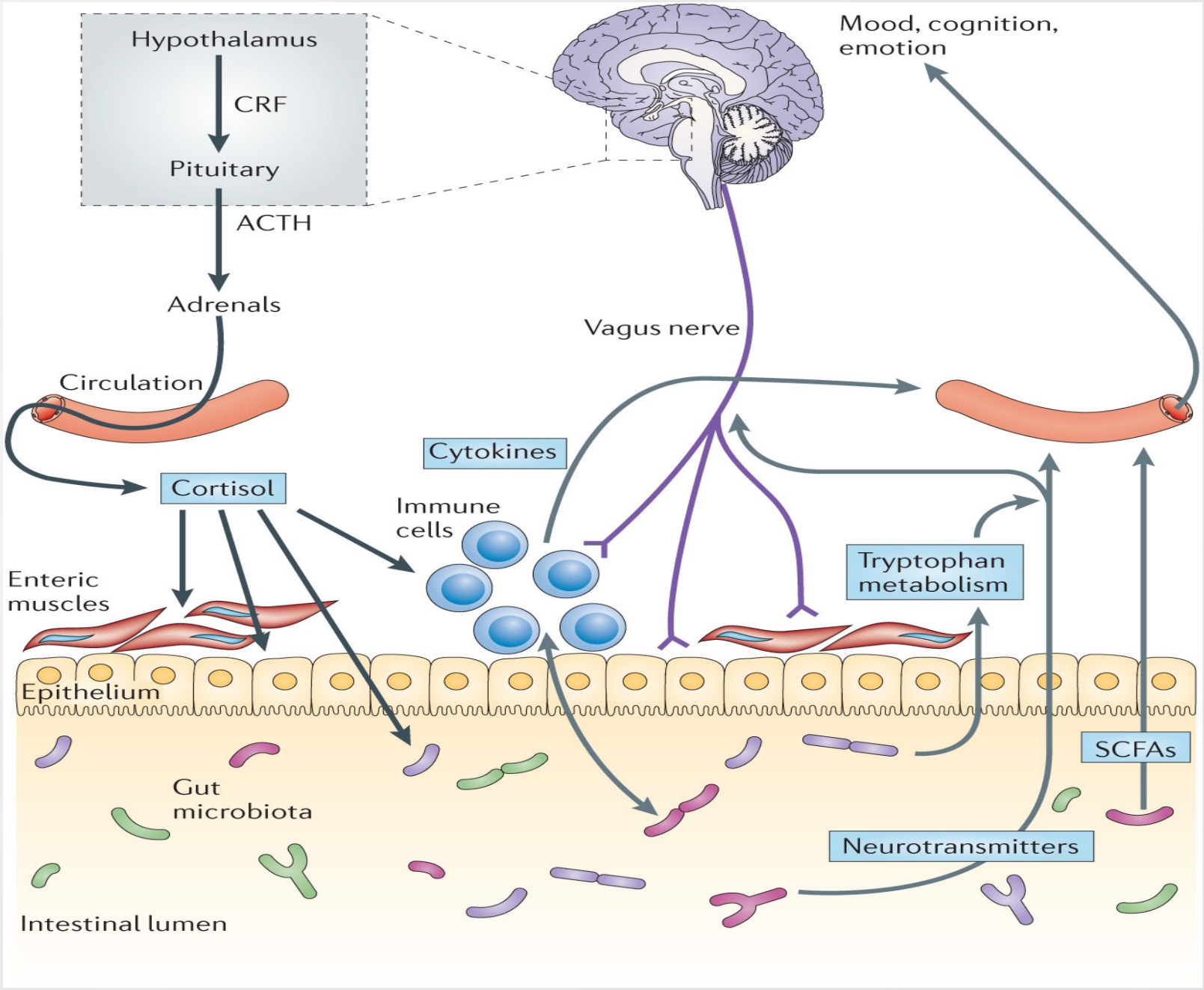
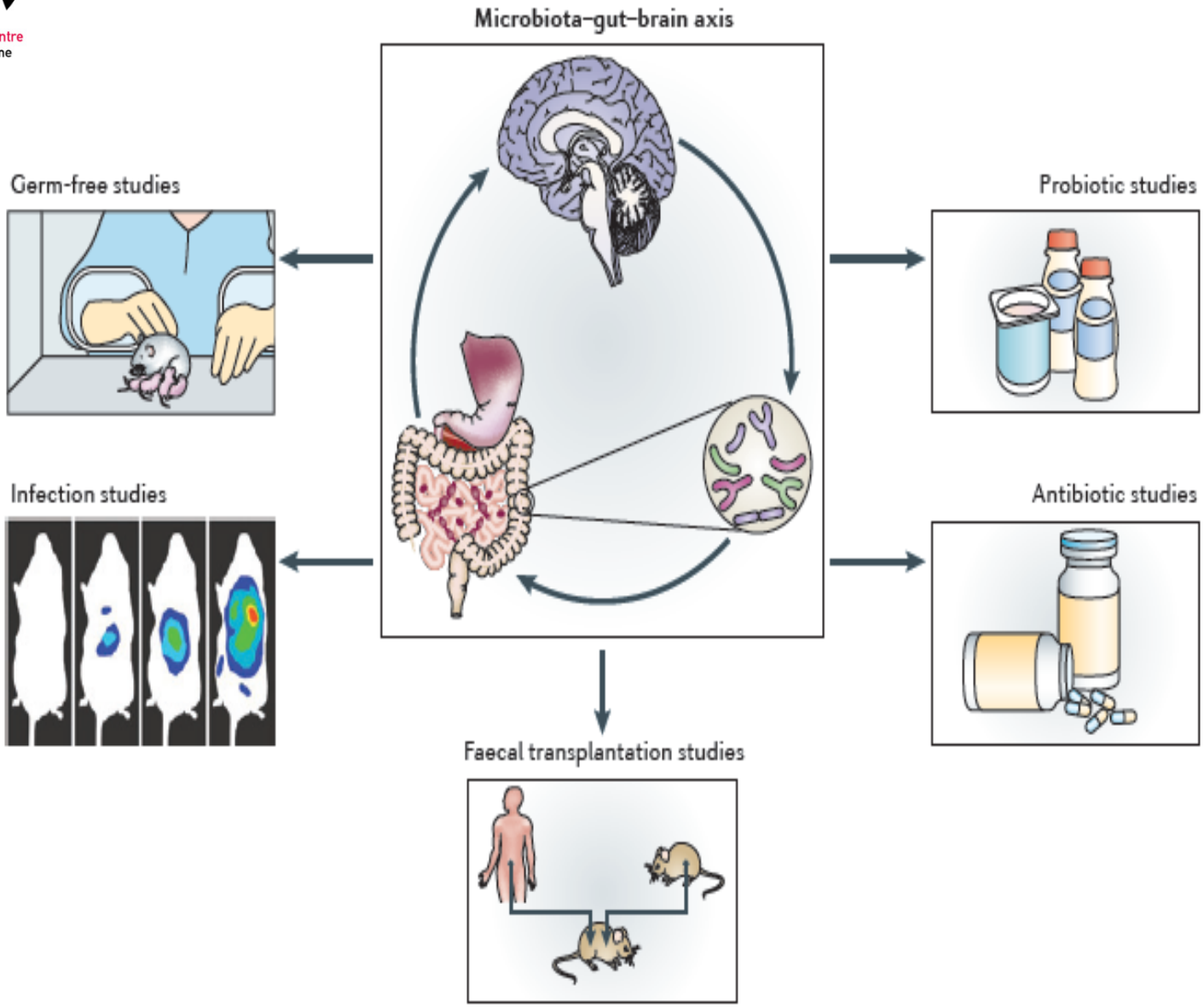
MICROBES, BRAIN AND BEHAVIOUR

Ted Dinan
Alimentary Pharmabiotic Centre
University College Cork
Ireland

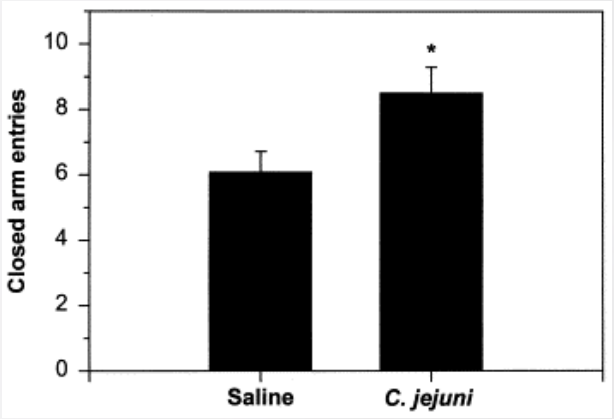


Cryan & Dinan, Nature Nurosci Rev 2012

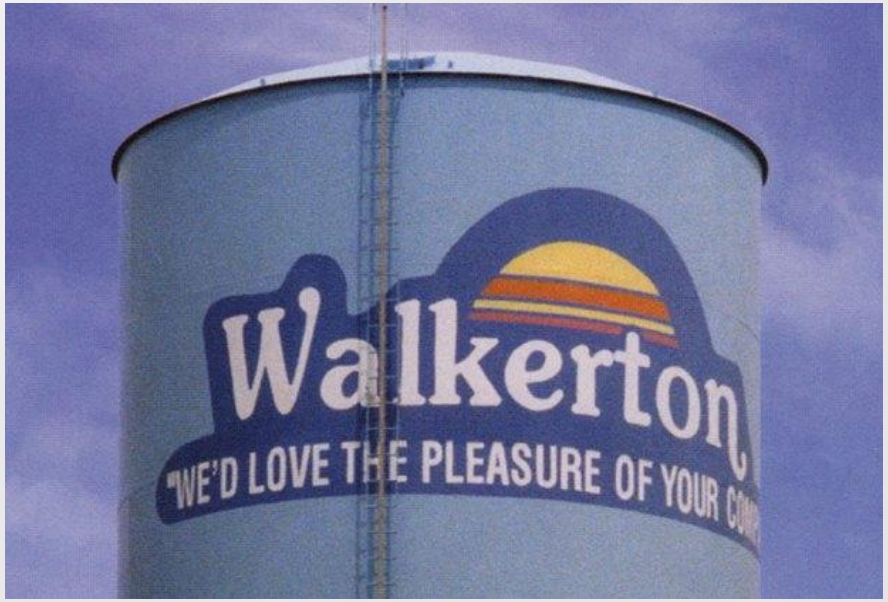


Cryan and Dinan, Nature Neurosci Rev (2012)

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

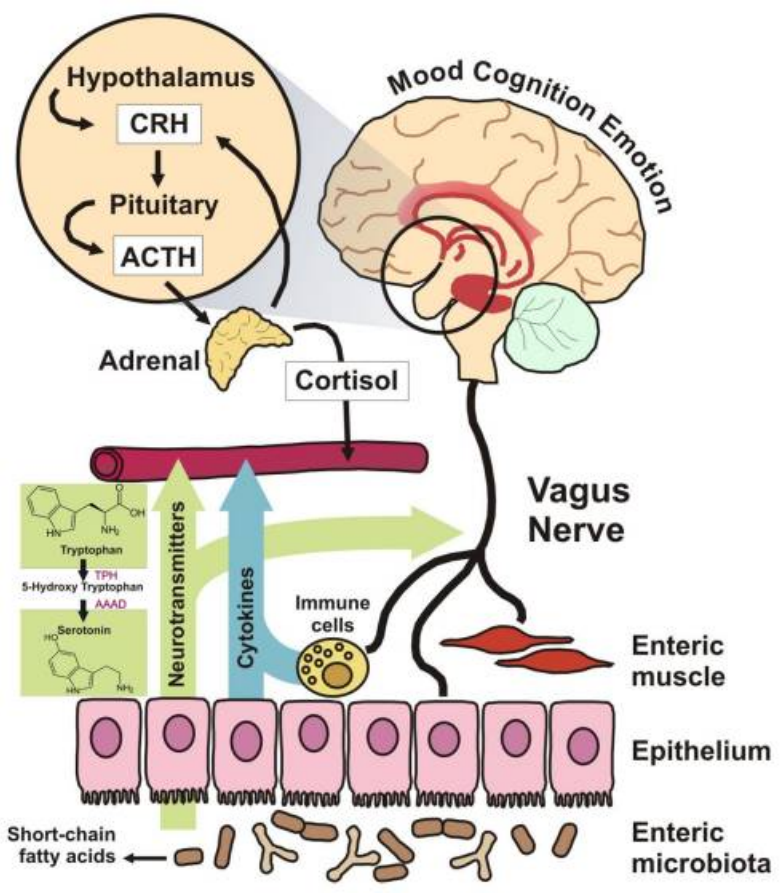
? Neurodegenerative disorders

? Neurodevelopmental disorders

T.J. Borody and A. Khoruts Nat Rev Gastroenterol Hepatol. 2011

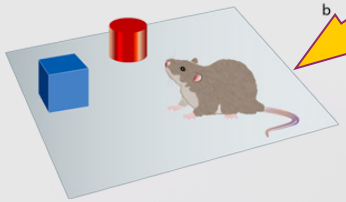
Germ-free in early life – effects on the social brain?

Microbiome-gut-brain axis

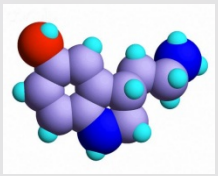


Germ-free mouse

Altered gut-brain communication

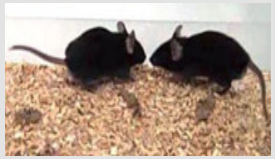


↓ memory



↑ brain serotonin

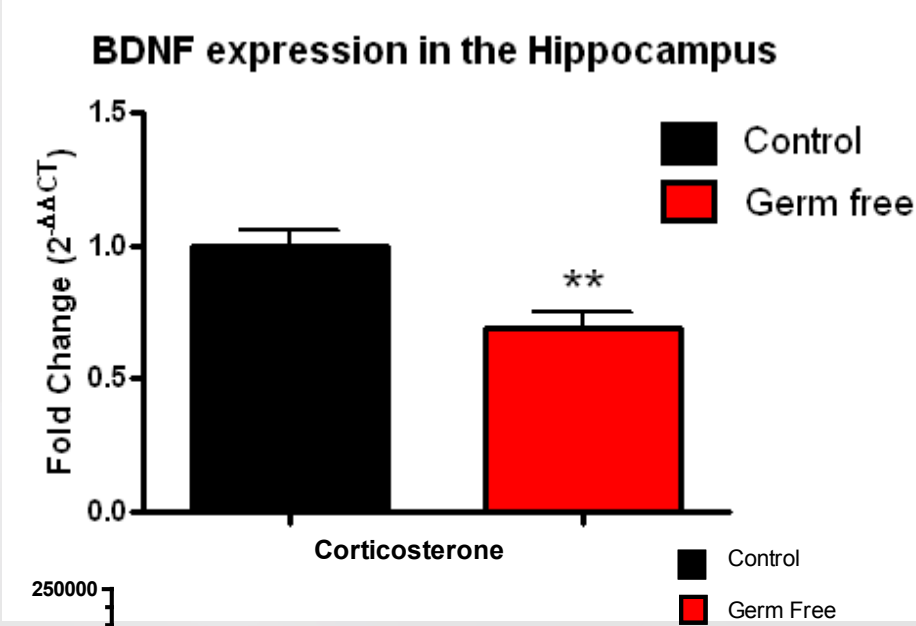
?



Altered social behaviours??

Decreased BDNF and Exaggerated Stress Response

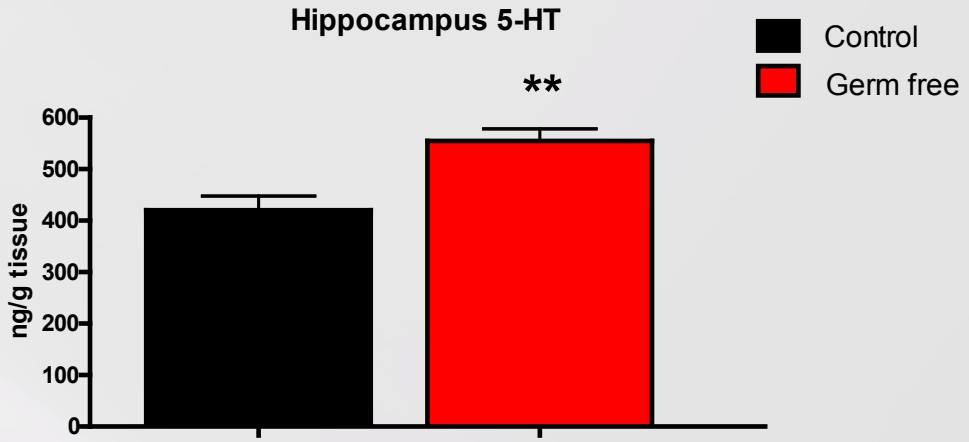
- BDNF is a neurotrophin supporting neuronal survival/growth
- Decreased BDNF in germ free compared to control animals
- Altered stress response



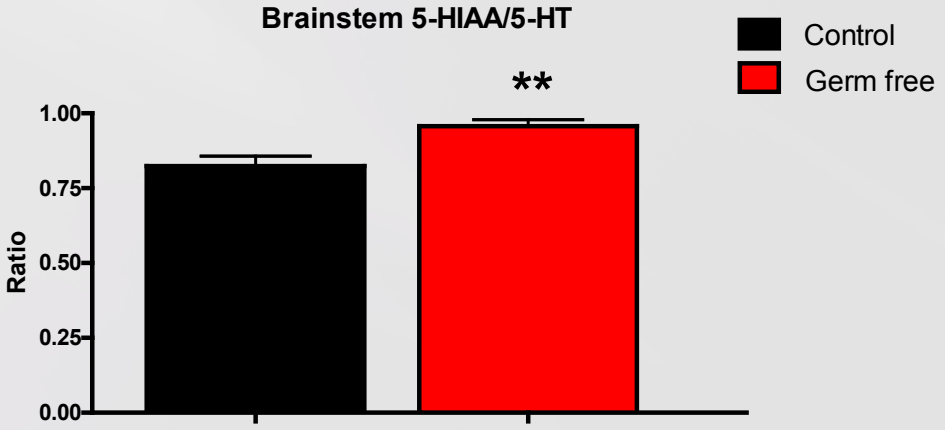
Grenham et al., 2011

Altered CNS Serotonergic Function

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



- Increased 5-HT turnover in brainstem



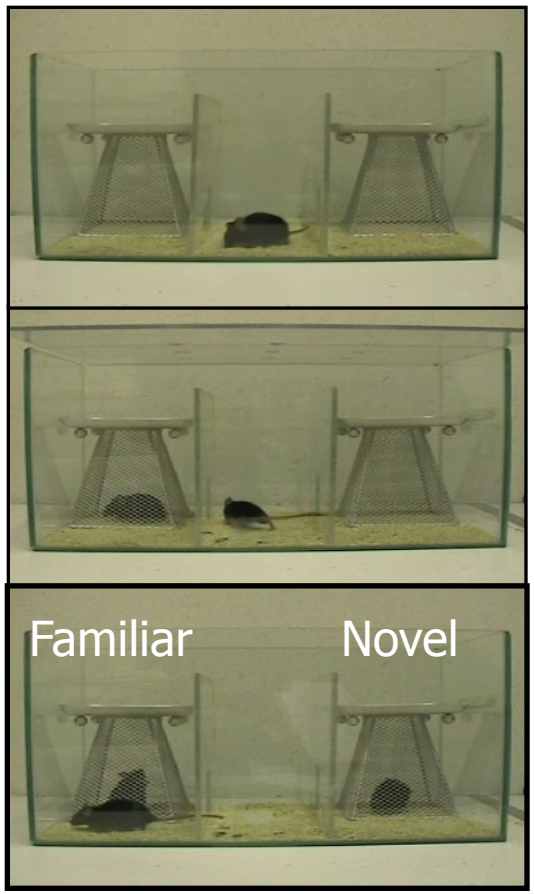
Clarke et al, 2012

Three-chamber sociability test

1.

2.

3.



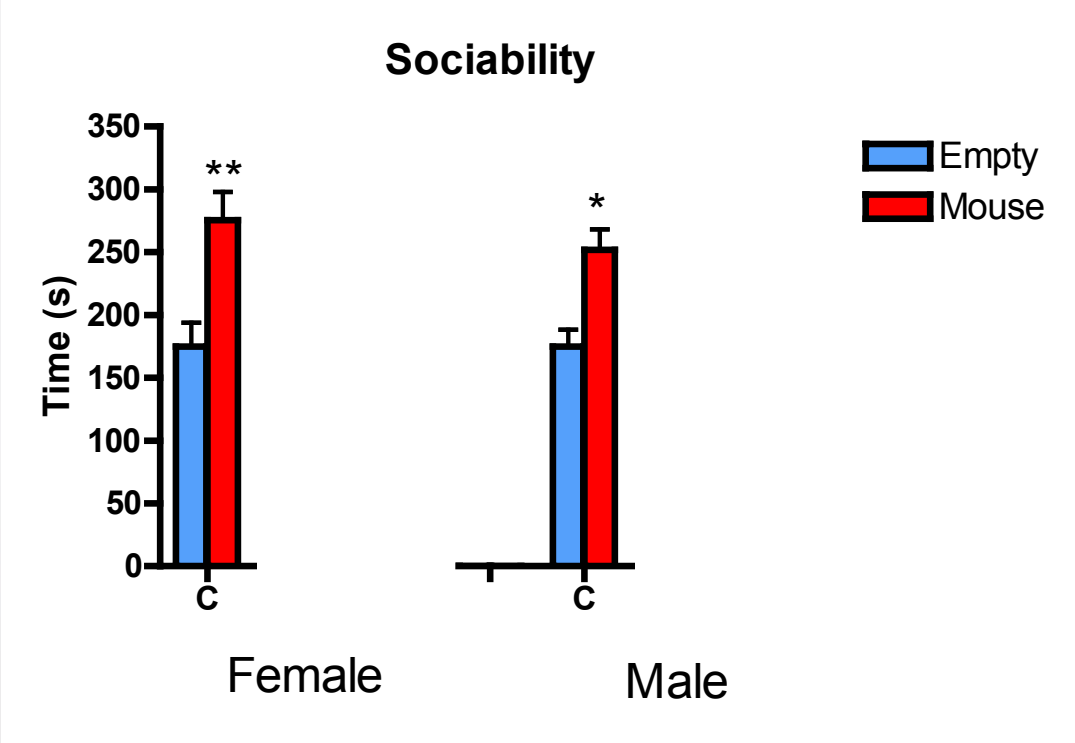
Habituation: exploration of 3 chambered box (10 mins).

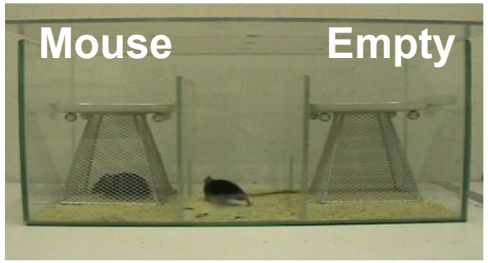
Sociability: does the test mouse spend more time in the chamber containing the mouse or in the opposite empty chamber?

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

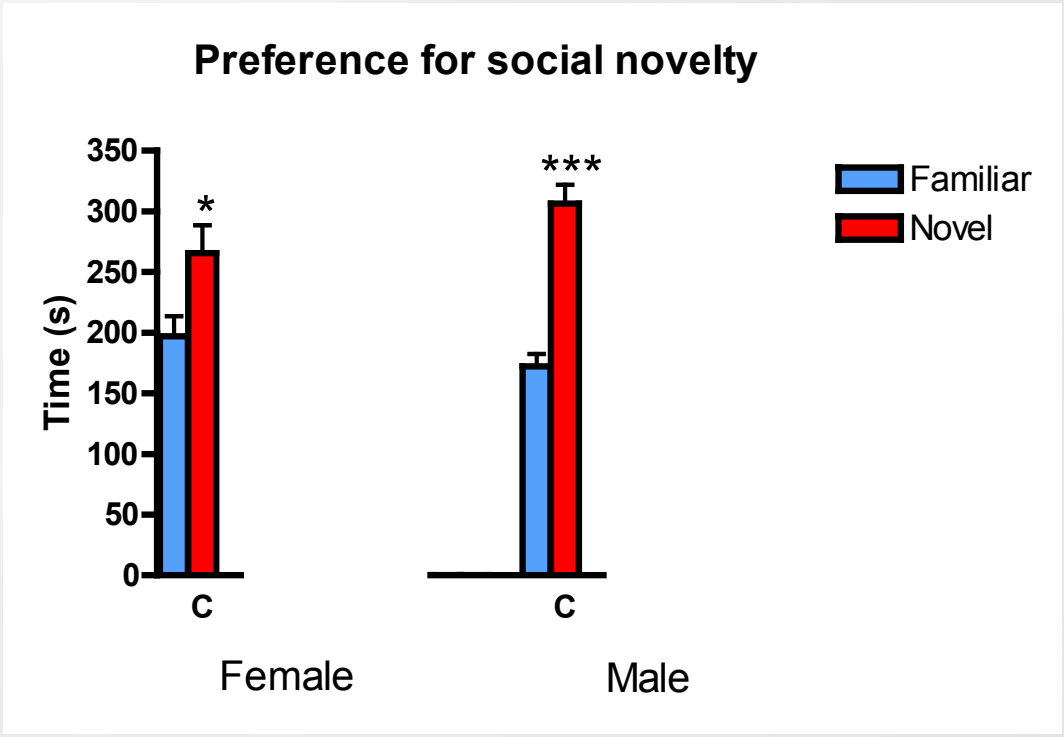


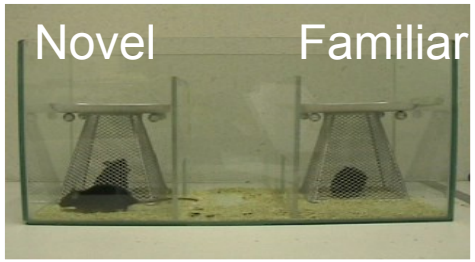


Conventional

Germ Free

Germ free effects on preference for social novelty

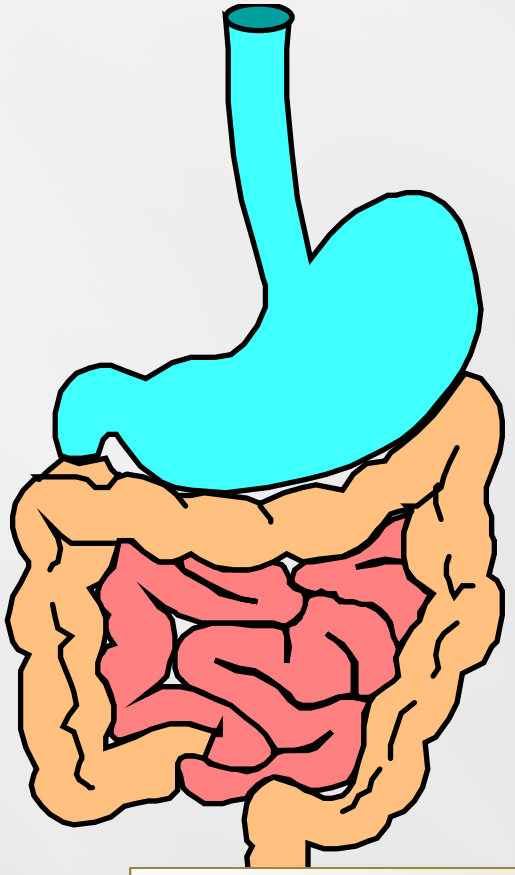




Conventional

Germ Free

Microbiota: Which species is the most effective neurochemical producer?



Jejunum: 10^{3-4}

Terminal Ileum: 10^{7-9}

Colon: 10^{10-12}

400–500 species including:

Bacteroides
Eubacterium
Peptostreptococcus
Bifidobacterium
Ruminococcus
Bacillus
Fusobacterium
Clostridium
Lactobacillus
Enterococcus
Enterobacter

Anaerobes >> Aerobes

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

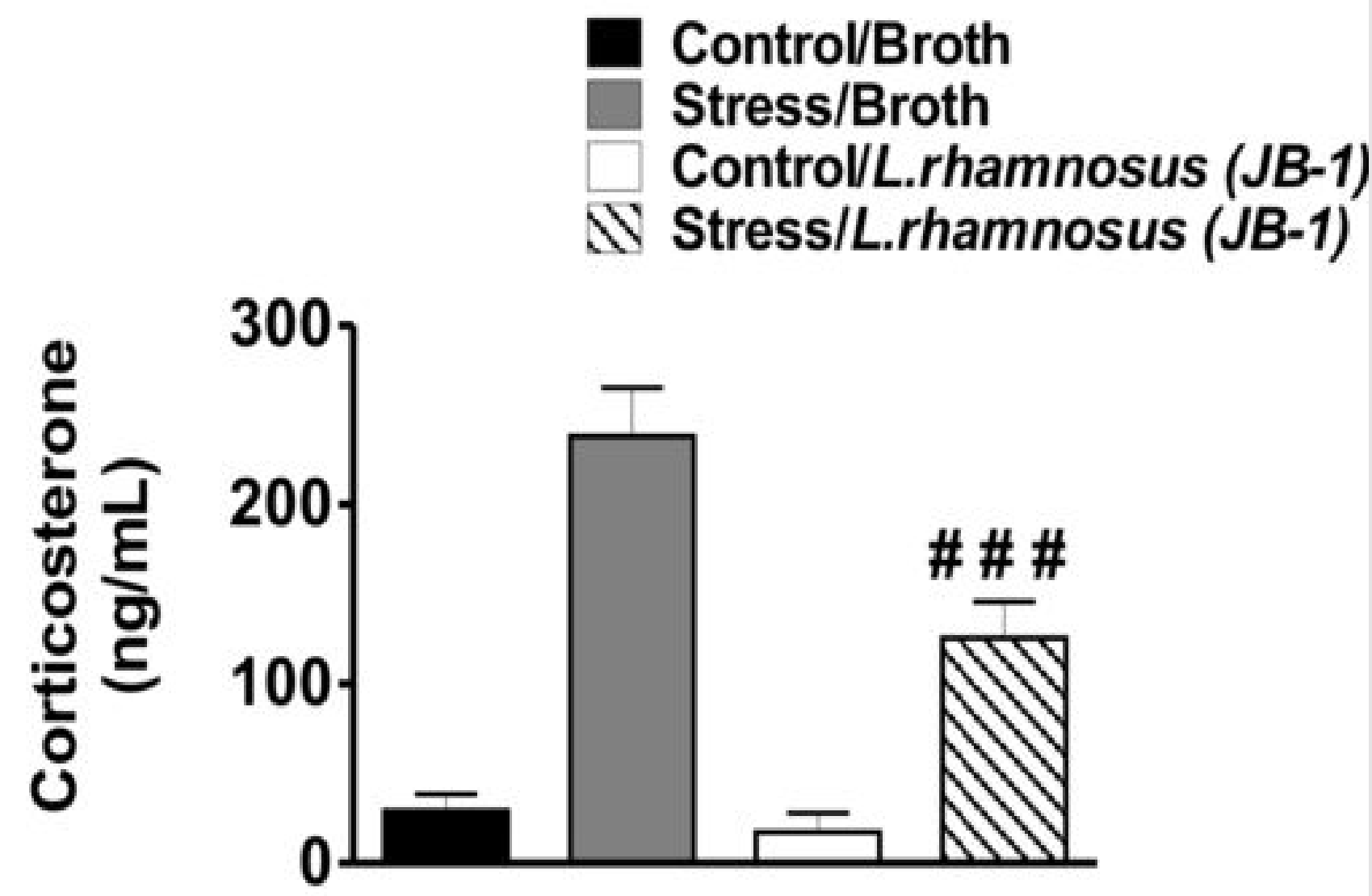
What neurotransmitters can be modulated by bacteria?

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

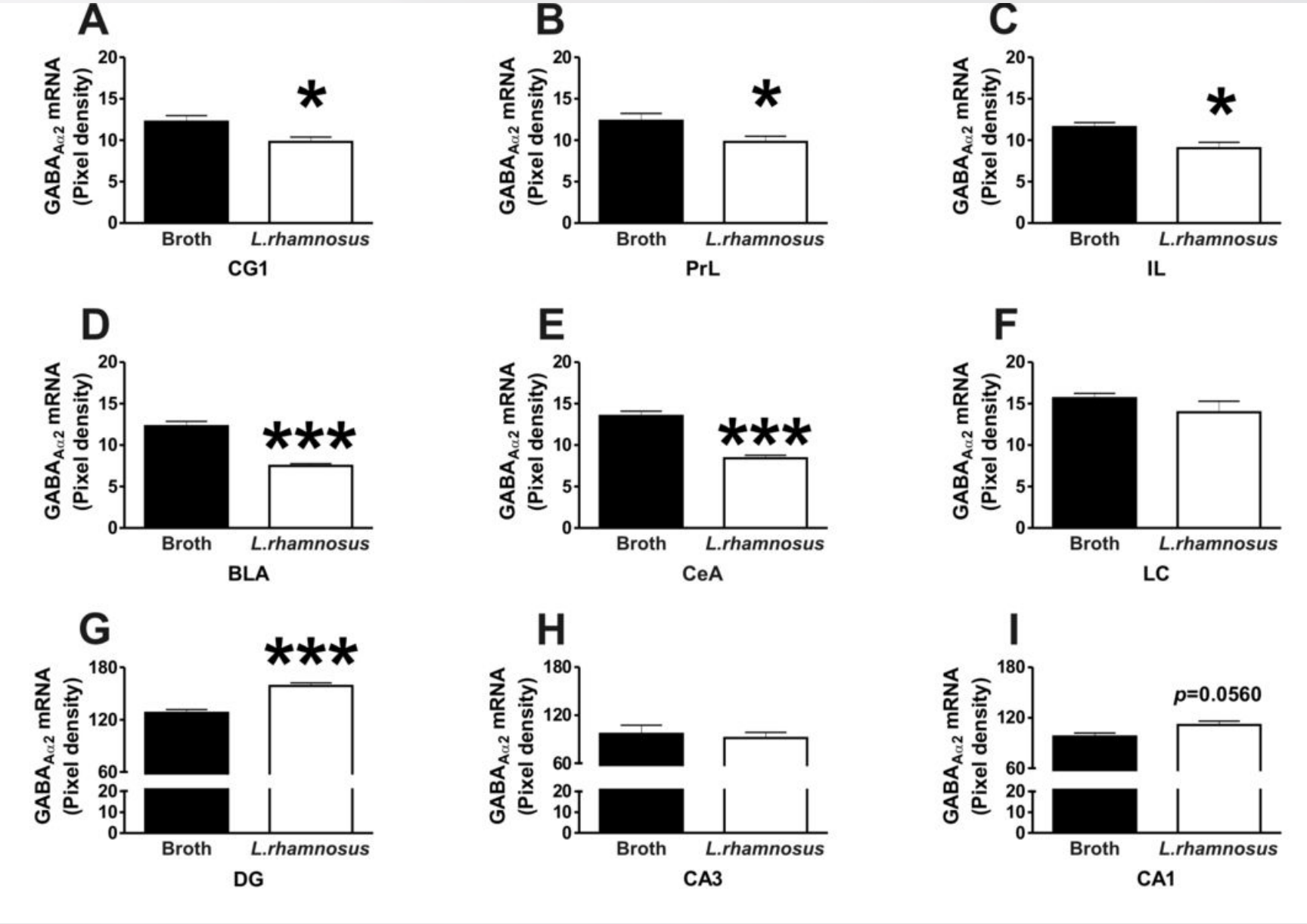
Bif. Infantis increases plasma tryptophan levels and thereby modulates 5HT

Lactobacillus rhamnosus alters central GABA receptor expression

Lactobacillus strain, GABA receptor expression and behaviour



Bravo et al, PNAS, 2011


A
GABA$_{A\alpha2}$ mRNA (Pixel density)
20
15
10
5
0
*
Broth
L.rhamnosus
CG1
B
GABA$_{A\alpha2}$ mRNA (Pixel density)
20
15
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5
0
*
Broth
L.rhamnosus
PrL
C
GABA$_{A\alpha2}$ mRNA (Pixel density)
20
15
10
5
0
*
Broth
L.rhamnosus
IL
D
GABA$_{A\alpha2}$ mRNA (Pixel density)
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p=0.0560
Broth
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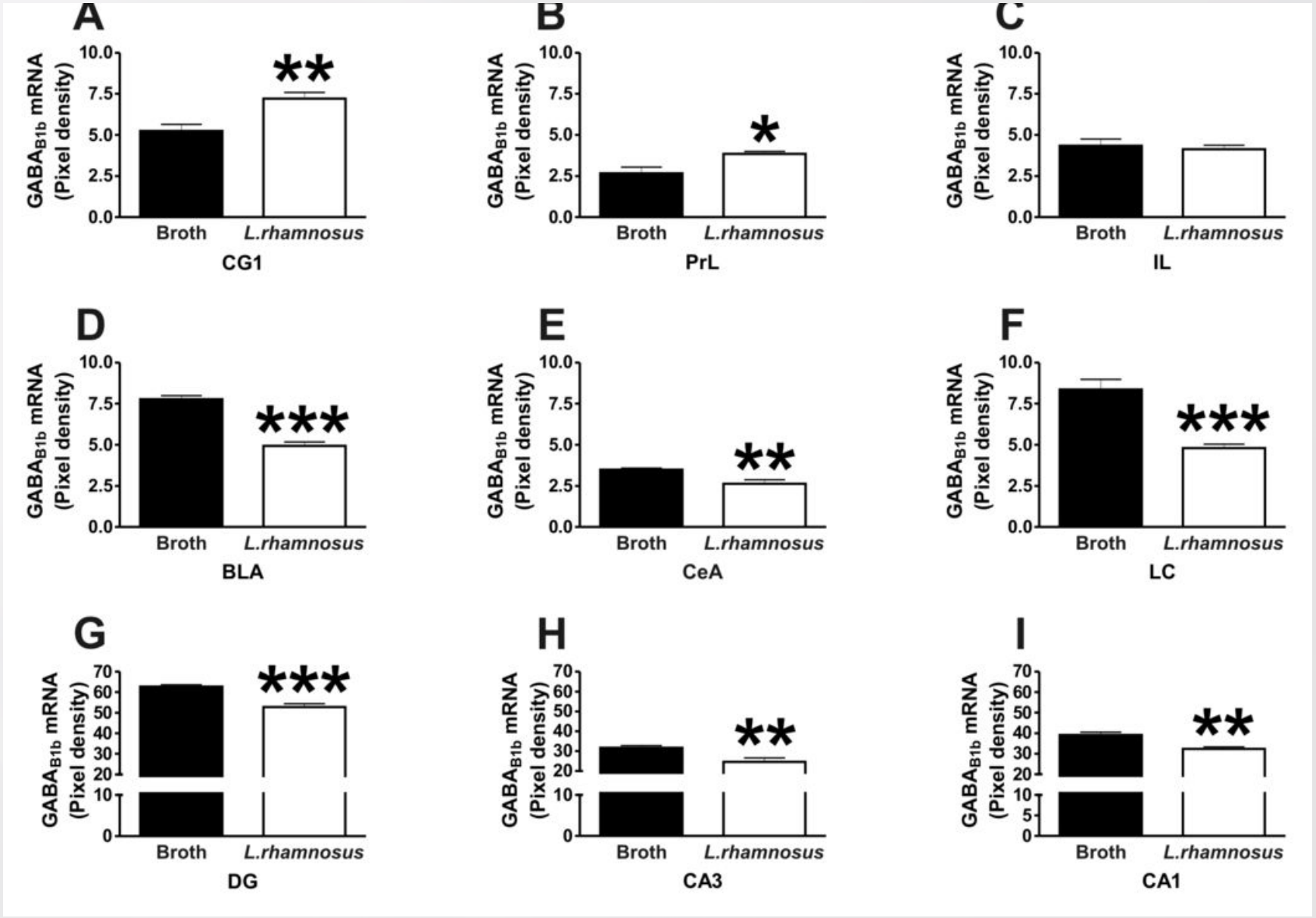
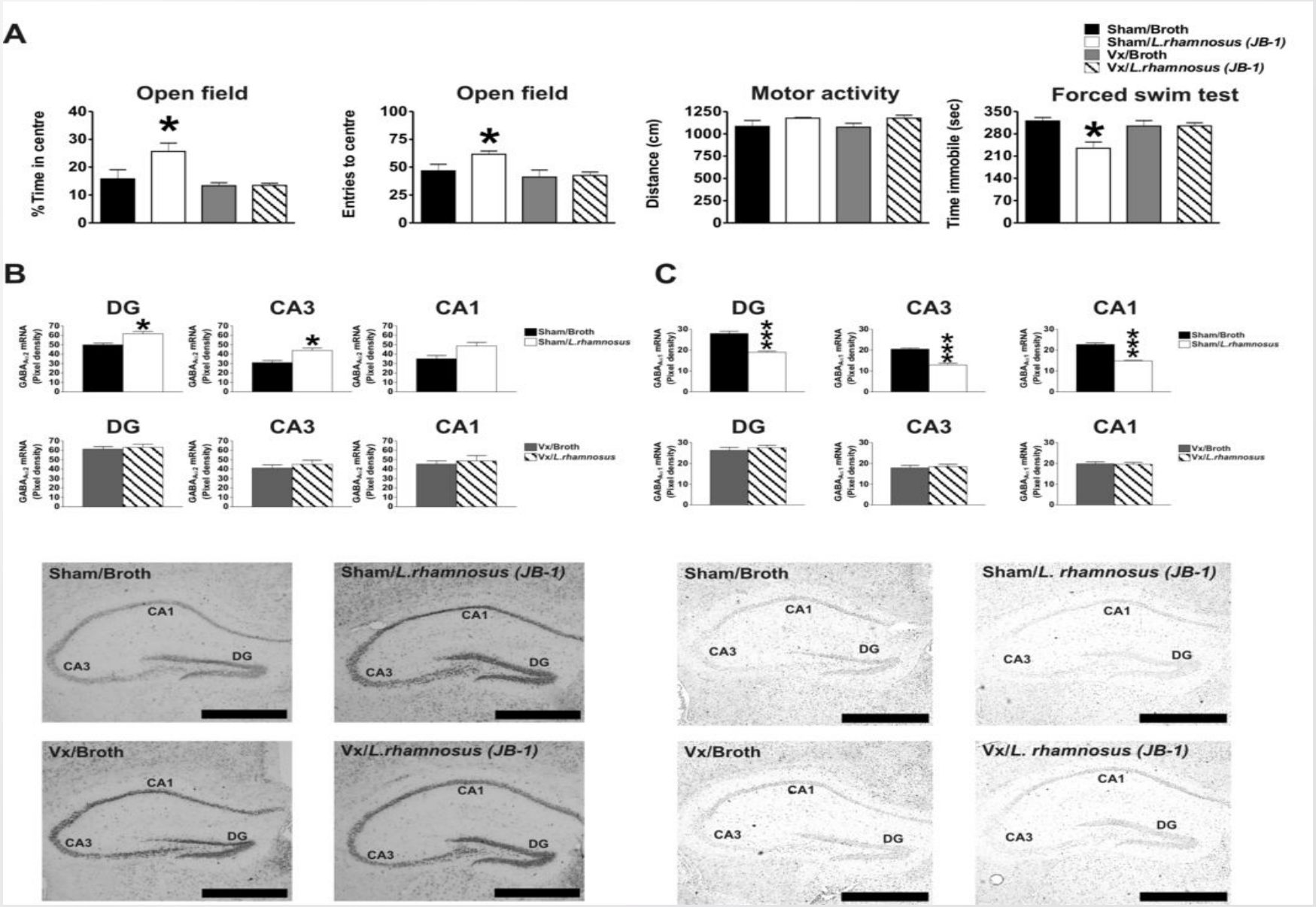
CA1



Bravo et al, PNAS, 2011

Effects of Vagotomy



Bravo et al, PNAS, 2011

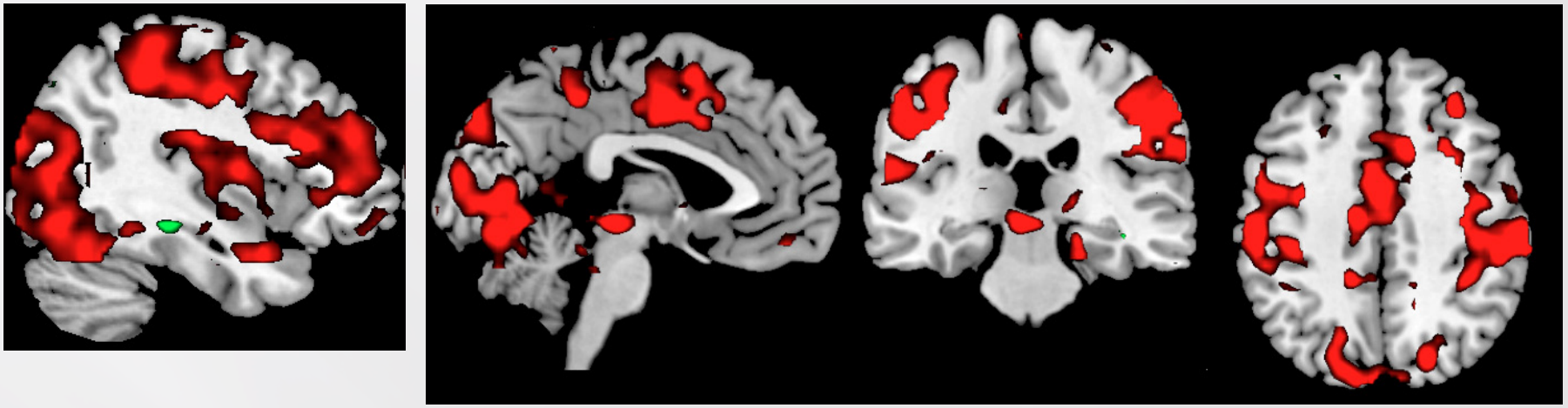
Can probiotic ingestion affect brain function in humans?

- 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)**

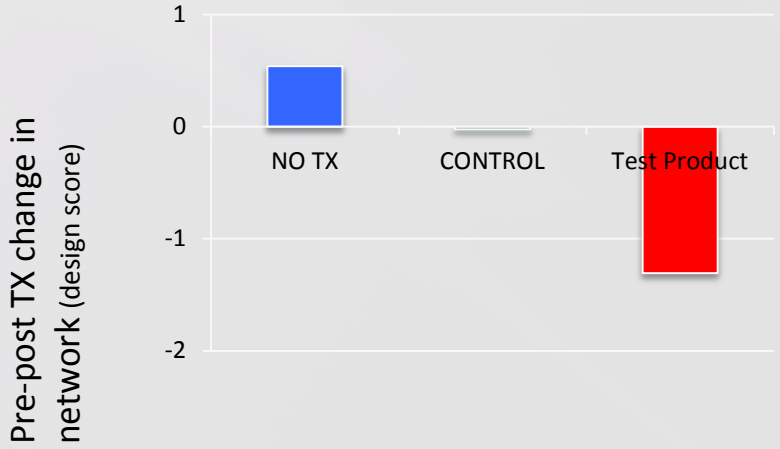
Tillisch, et al, Gastroenterology 2013

Intake of FMPP was associated with decreased connectivity of an extensive brain network including somato- and viscerosensory regions in response to the task



Network crossblock covariance 49%, P <.005

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



Tillisch, et al, Gastroenterology 2013

REVIEW BIOLOGICAL PSYCHIATRY 2013

Psychobiotics: A Novel Class of Psychotropic

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

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?