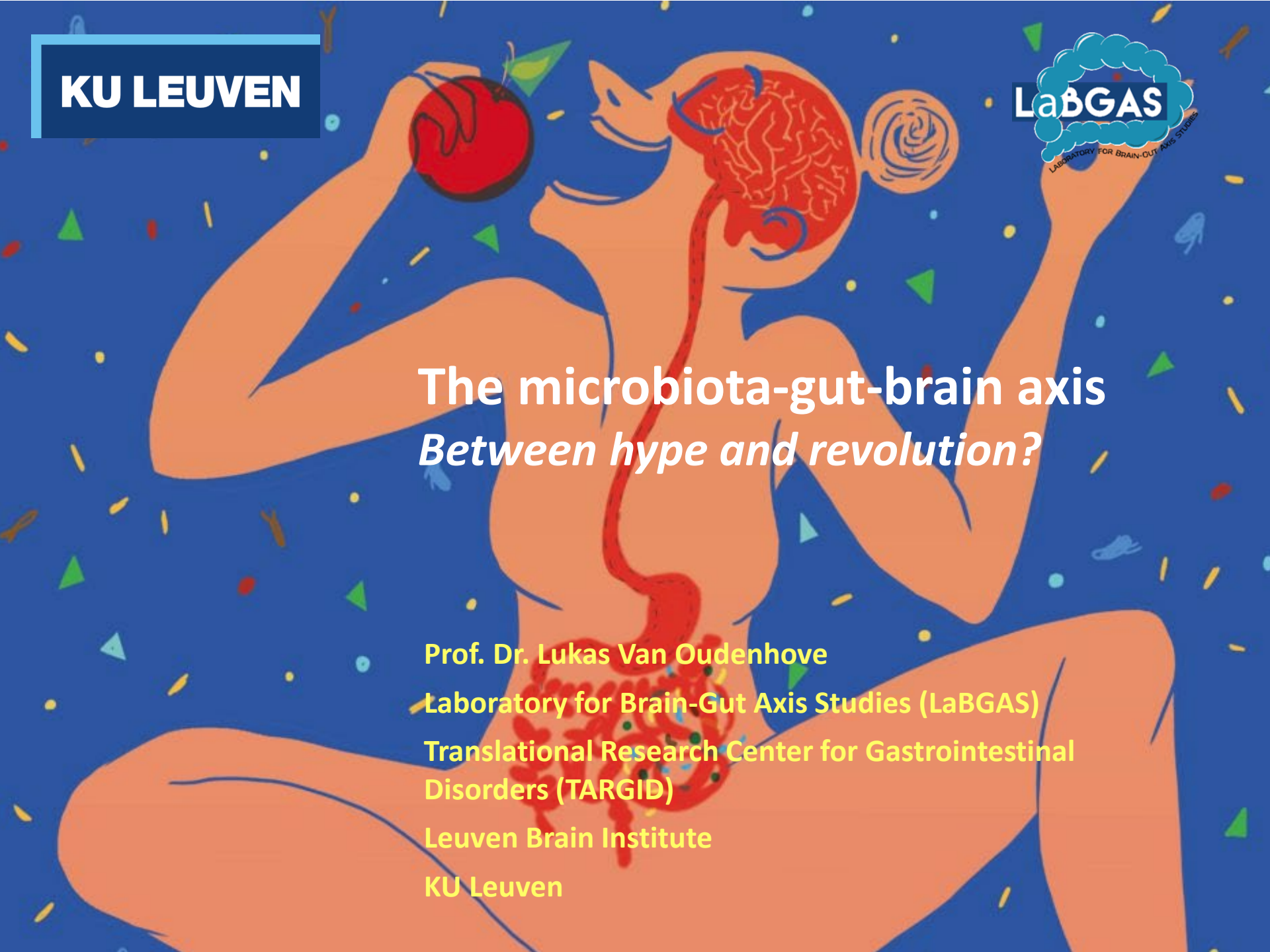


KU LEUVEN

LaBGAS
LABORATORY FOR BRAIN-GUT AXIS STUDIES

The microbiota-gut-brain axis
Between hype and revolution?

Prof. Dr. Lukas Van Oudenhove

Laboratory for Brain-Gut Axis Studies (LaBGAS)

Translational Research Center for Gastrointestinal Disorders (TARGID)

Leuven Brain Institute

KU Leuven

Microbiota-Gut-Brain Axis
The hype of the day?

KATHOLIEKE UNIVERSITEIT LEUVEN

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

John F. Cryan[1,2] and Timothy G. Dinan[1,3]

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 Tillisch[1,5]

REVIEW

Psychobiotics: A Novel Class of Psychotropic

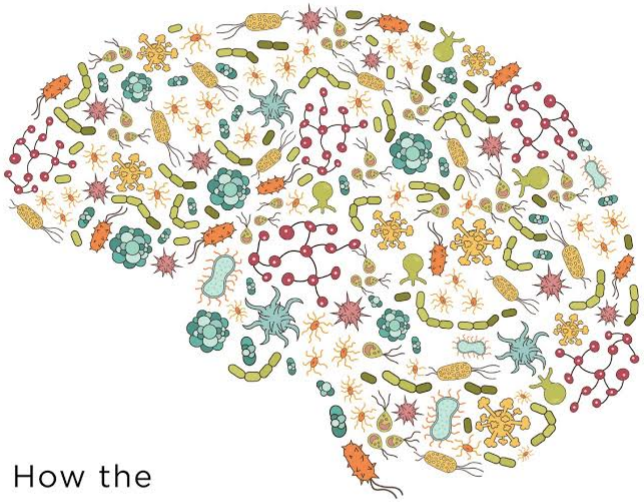
Timothy G. Dinan, Catherine Stanton, and John F. Cryan

Review

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

Karen-Anne McVey Neufeld[a], Pauline Luczynski[a], Clara Seira Oriach[a,b], Timothy G. Dinan[a,b], John F. Cryan[a,c,*]

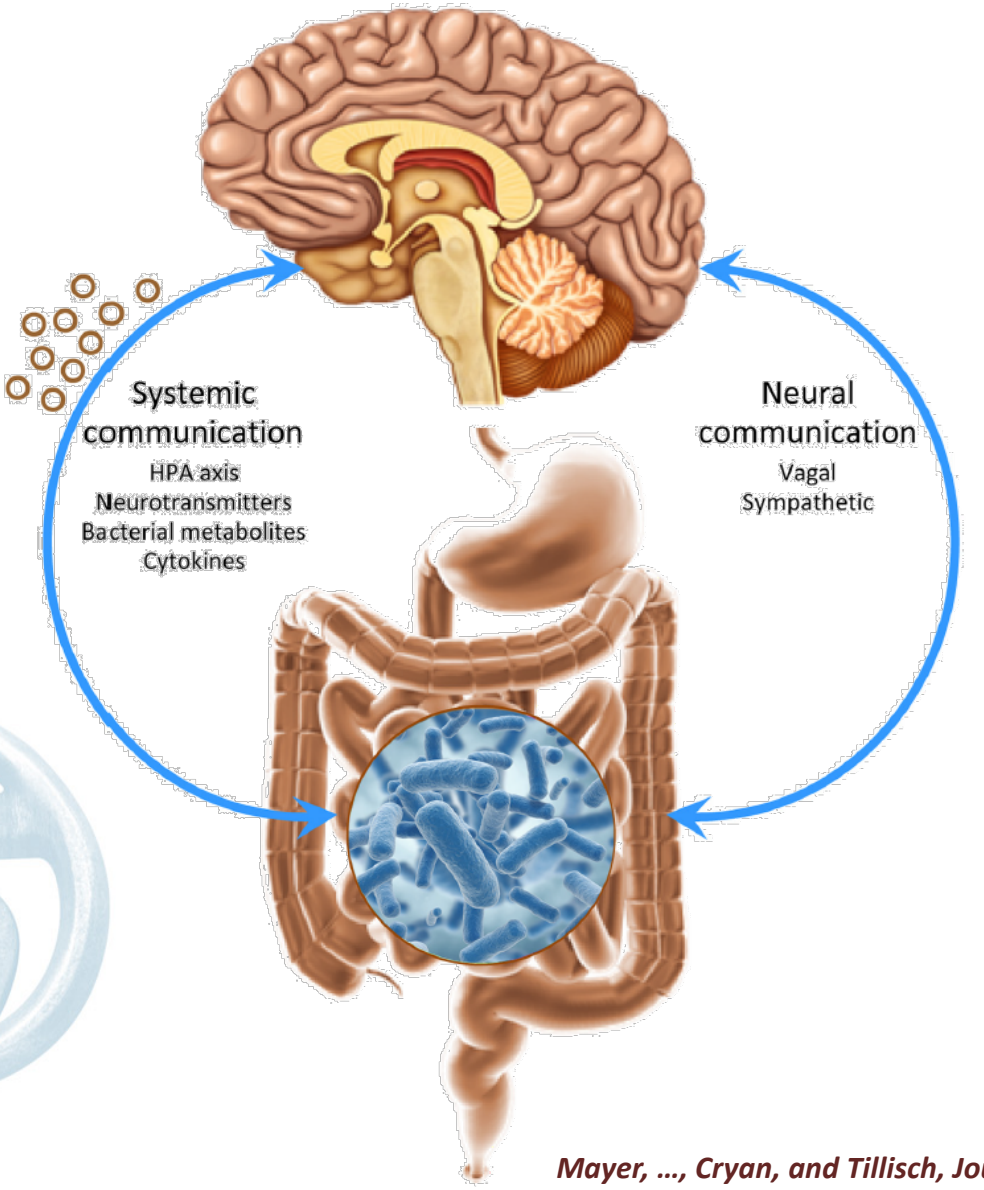
THE Mind-Gut CONNECTION



How the Hidden Conversation Within Our Bodies Impacts Our Mood, Our Choices, and Our Overall Health

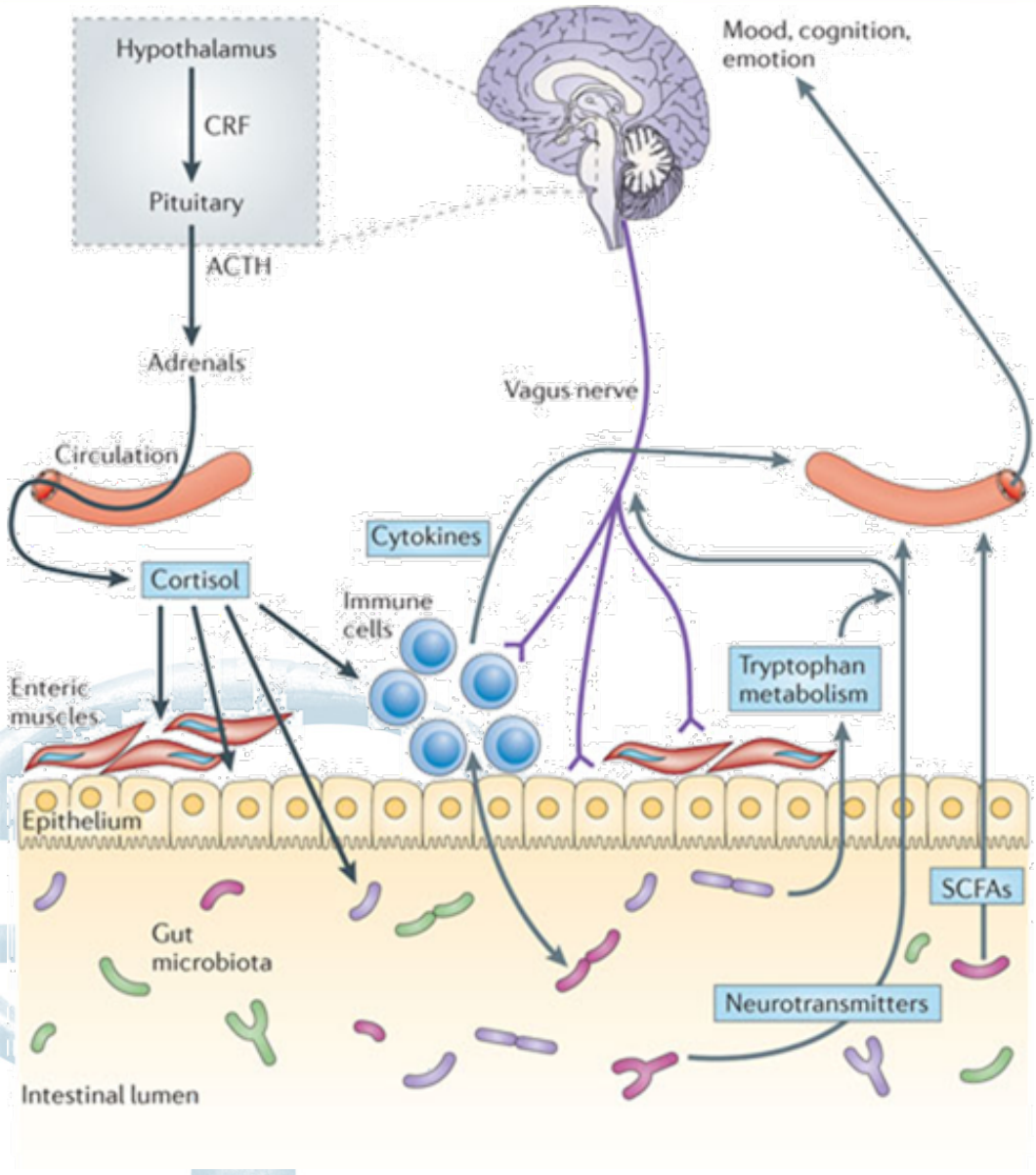
Emeran Mayer, MD

Microbiota-Gut-Brain Axis
What?



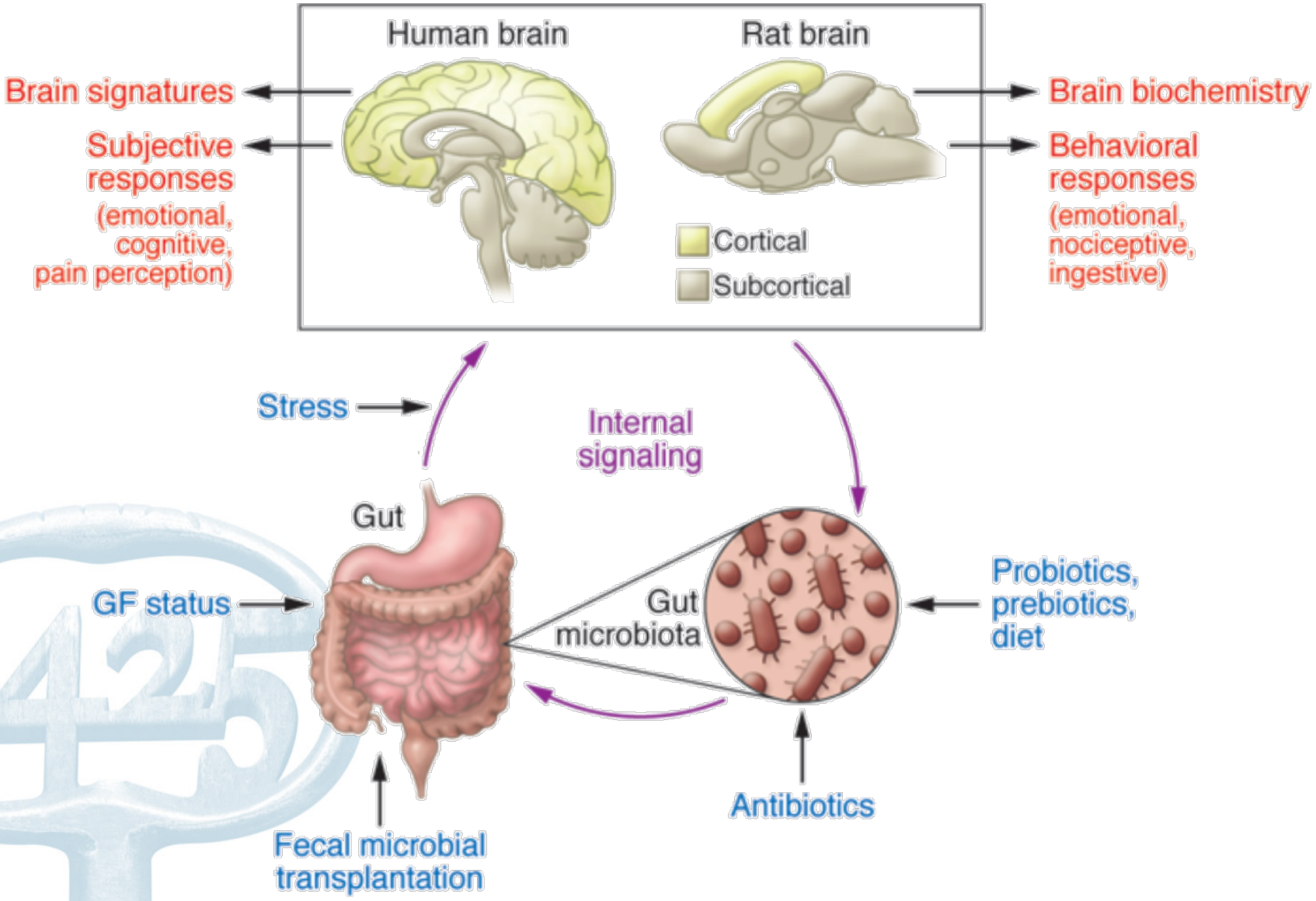
Mayer, …, Cryan, and Tillisch, Journal of Neuroscience 2014

Microbiota-gut-brain axis
Signaling mechanisms



Cryan & Dinan, Nature Reviews Neuroscience 2012

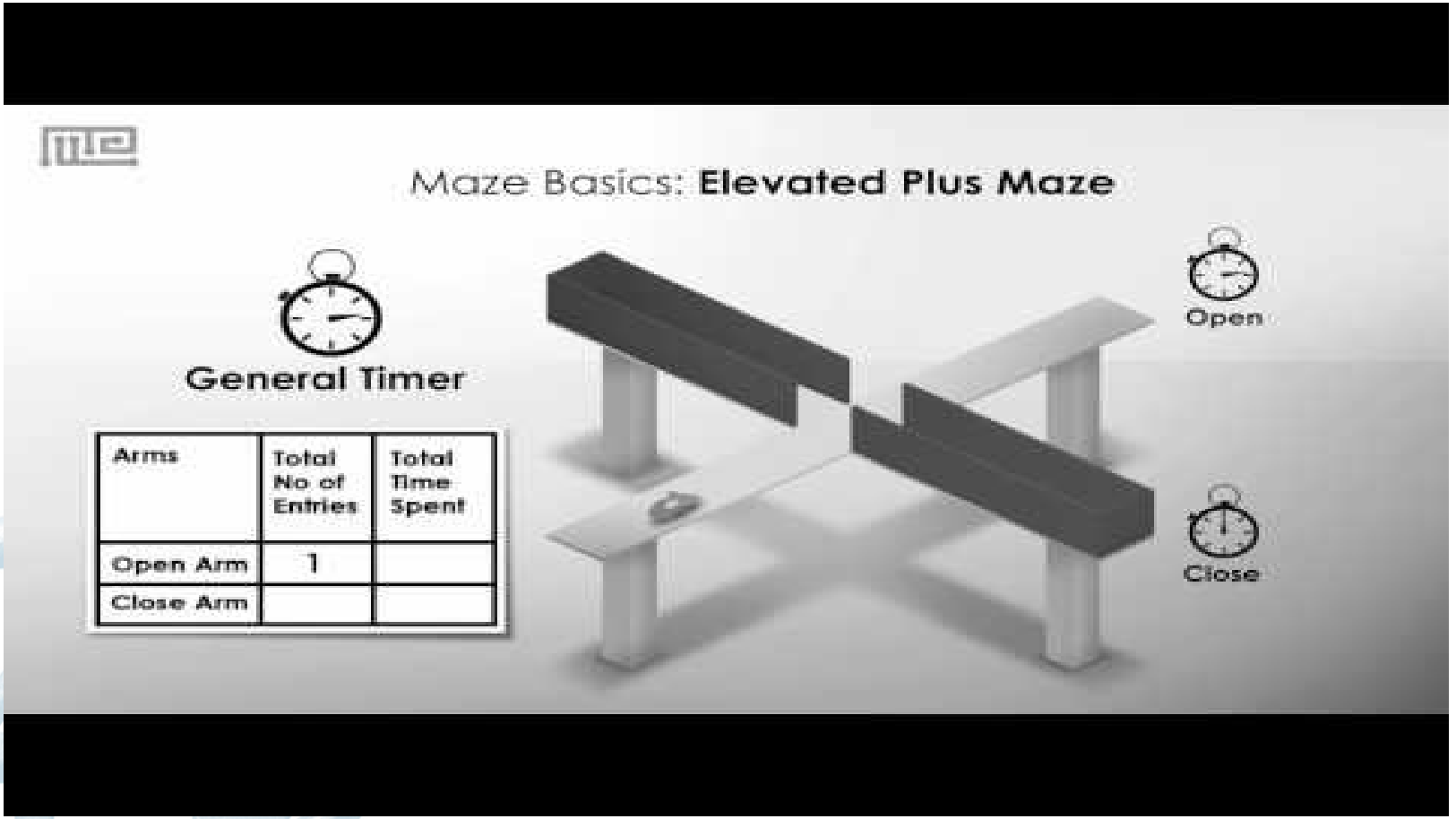
Microbiota-gut-brain axis
How to study?



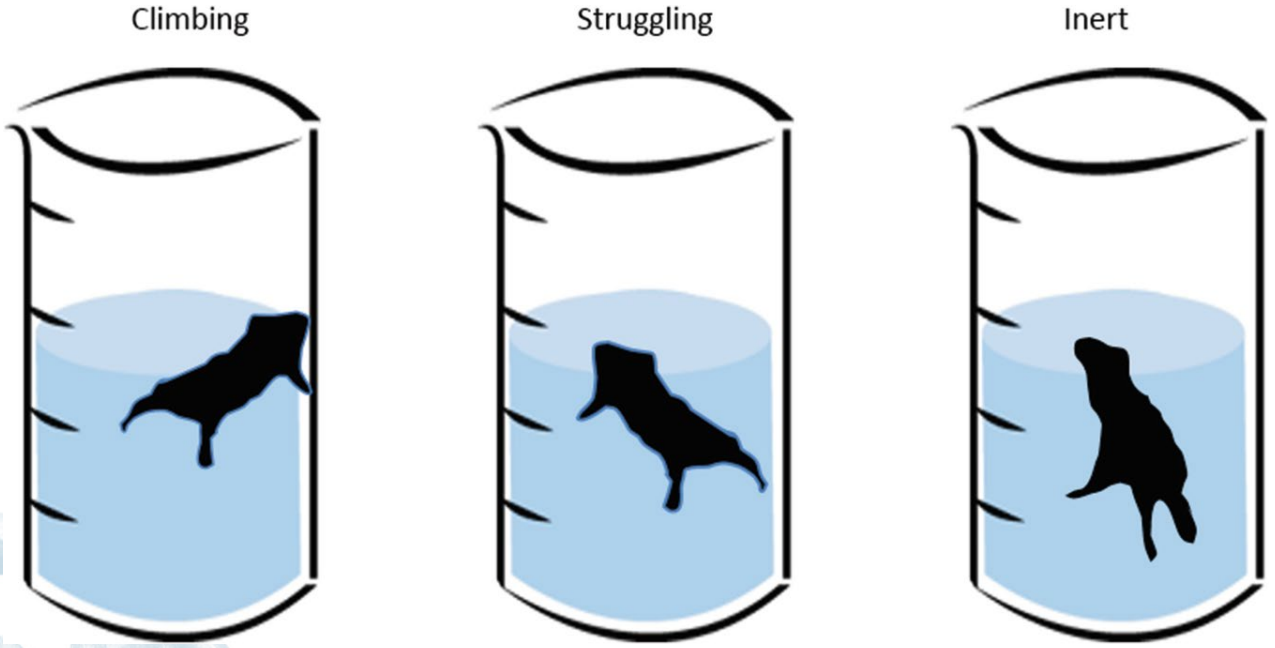
Microbiota-gut-brain axis
definitions

- **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 micro-organisms conferring a health benefit"
- **"psychobiotics"**
  - either of the above conferring a health benefit *in patients with psychiatric illness*
- **fecal microbiota transplantation**

How to test anxious behaviour in mice? elevated plus maze test



How to test depressive behaviour in mice? forced swim test

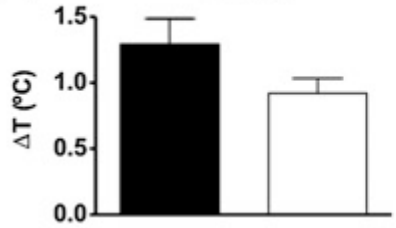


Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

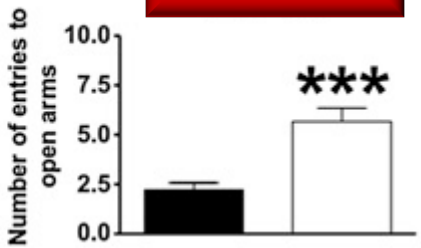
Javier A. Bravo[a,1], Paul Forsythe[b,c,1], Marianne V. Chew[b], Emily Escaravage[b], Hélène M. Savignac[a,d], Timothy G. Dinan[a,e], John Bienenstock[b,f,2], and John F. Cryan[a,d,g,2]

16050–16055 | PNAS | September 20, 2011 | vol. 108 | no. 38

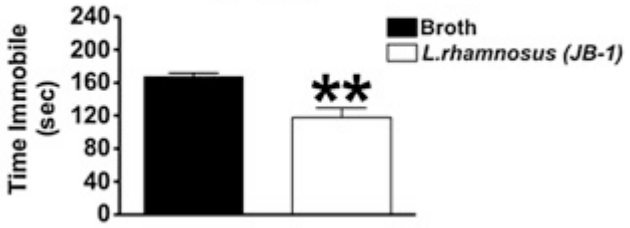
stress-induced hyperthermia



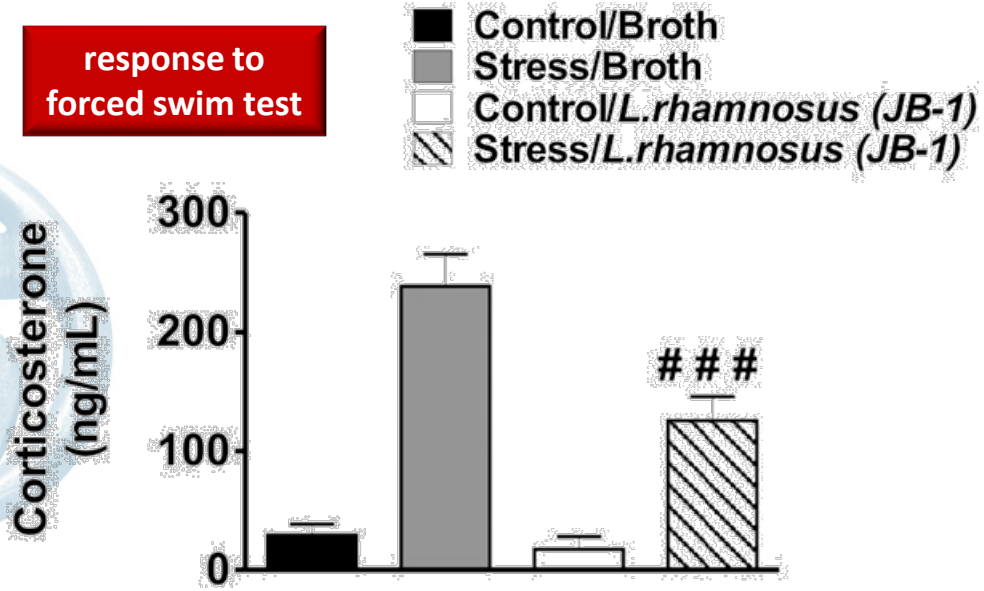
elevated plus maze



forced swim test



response to forced swim test



Bravo, Forsythe et al, Proceedings of the National Academy of Sciences USA 2011

Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

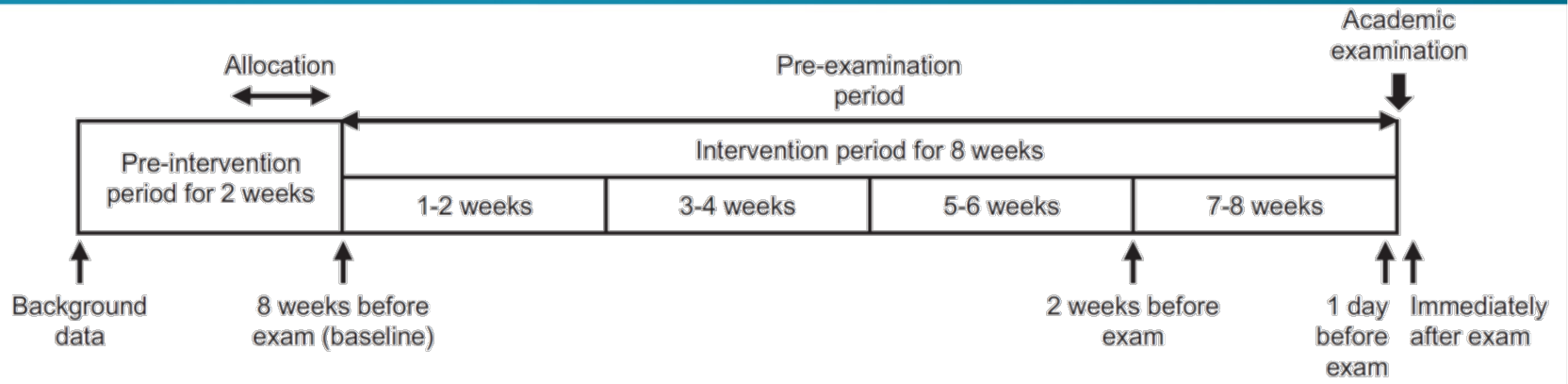
Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects

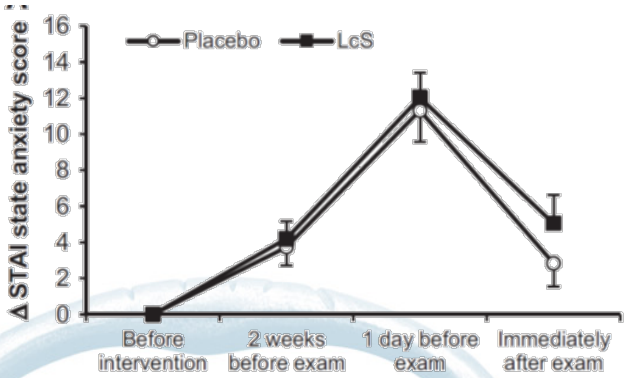
John R. Kelly [a,b], Andrew P. Allen [a,b], Andriy Temko [c], William Hutch [d], Paul J. Kennedy [a], Niloufar Farid [b], Eileen Murphy [e], Geraldine Boylan [d], John Bienenstock [f], John F. Cryan [a,g], Gerard Clarke [a,b], Timothy G. Dinan [a,b,*]

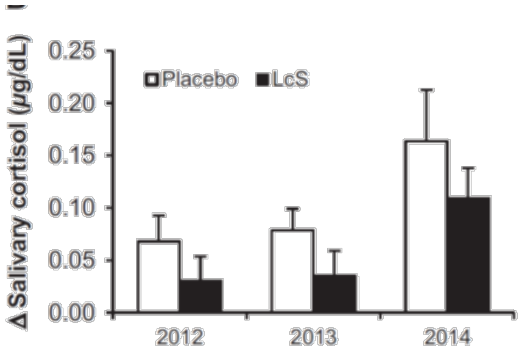
No effect on HPA axis response
No effect on inflammation
No effect on cognitive tasks

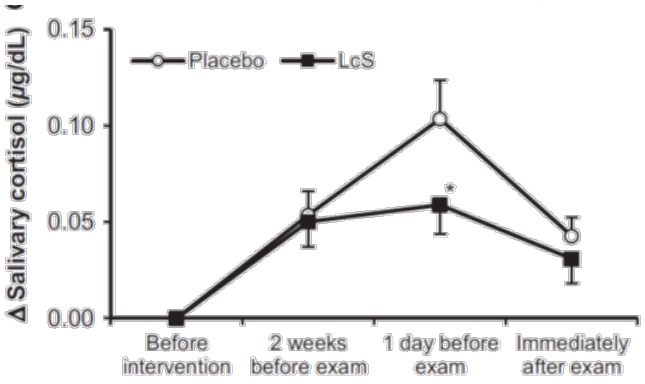
Bravo, Forsythe et al, Proceedings of the National Academy of Sciences USA 2011

Not lost in translation!
Lactobacillus Casei Shirota







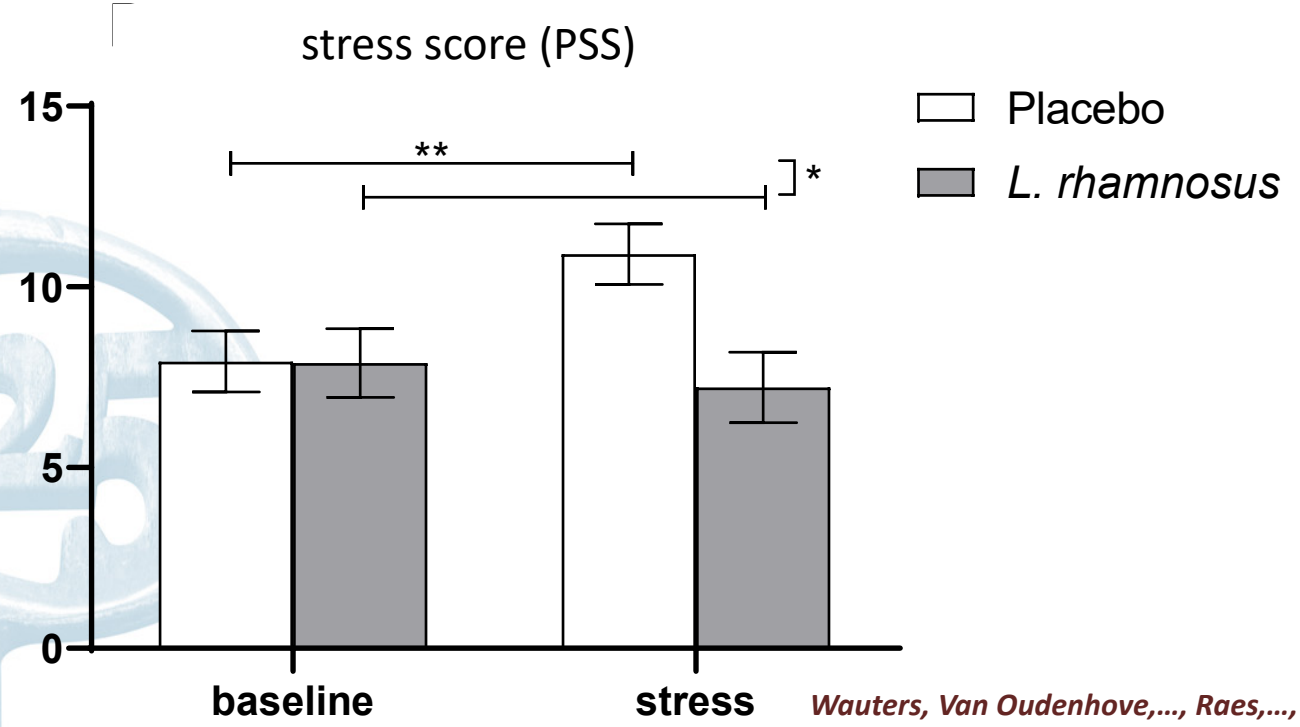




Takada et al, Neurogastroenterol Motil 2016

Not lost in translation!
Lactobacillus Rhamnosus CNCM-I 3690



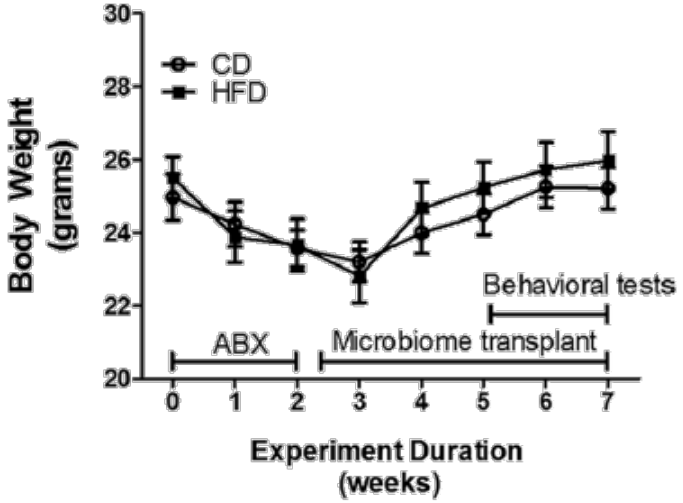


Wauters, Van Oudenhove,..., Raes,..., Vanuytsel, unpublished

Obese-type Gut Microbiota Induce Neurobehavioral Changes in the Absence of Obesity

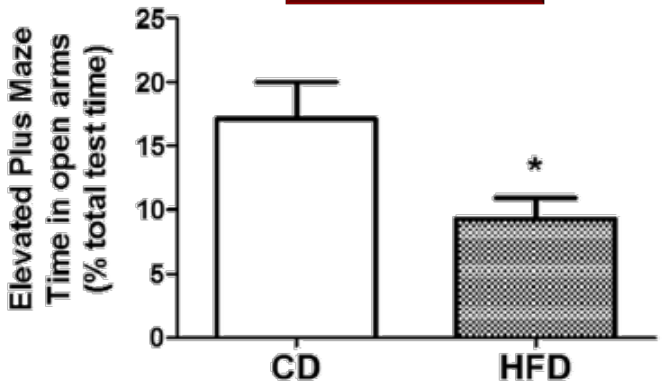
KATHOLIEKE UNIVERSITEIT LEUVEN

A

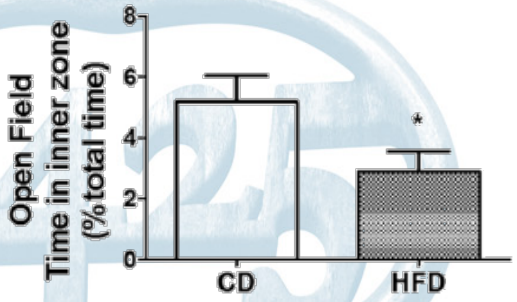


B

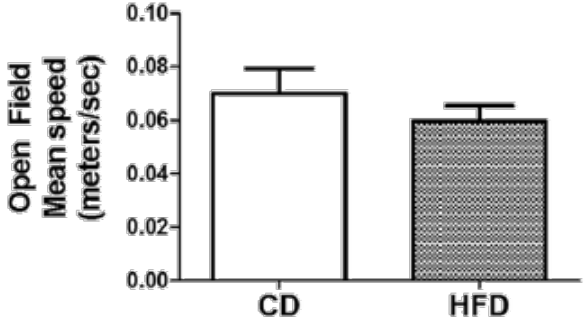
elevated plus maze

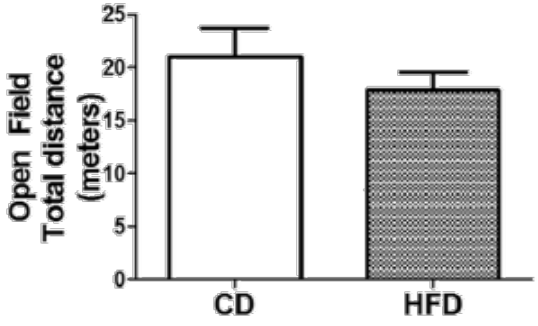


C



open field test

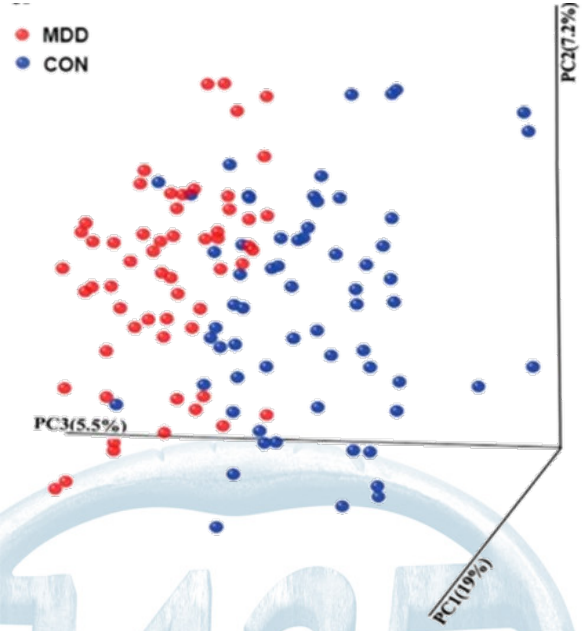


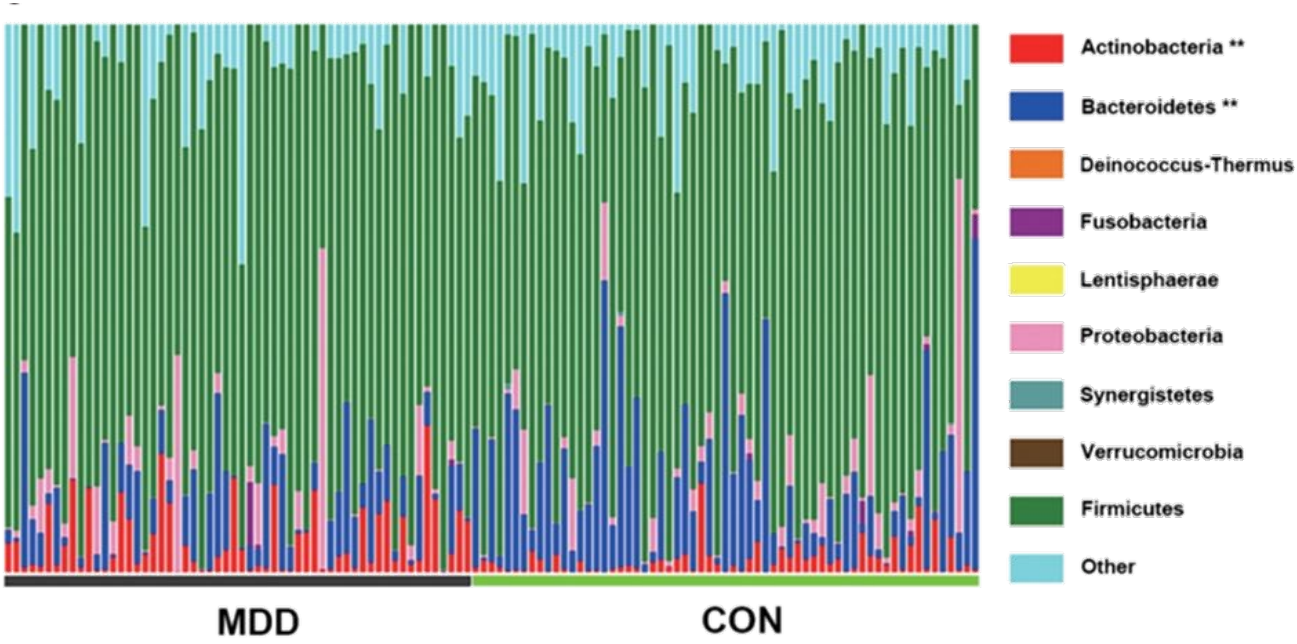


overall locomotor activity

Bruce-Keller et al, Biological Psychiatry 2015

Altered microbiota composition in major depressive disorder

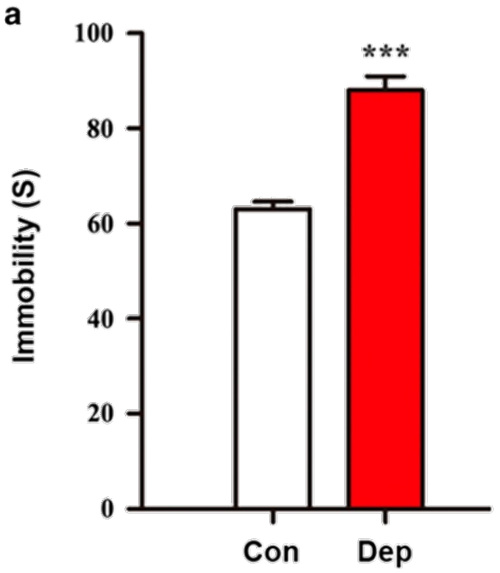


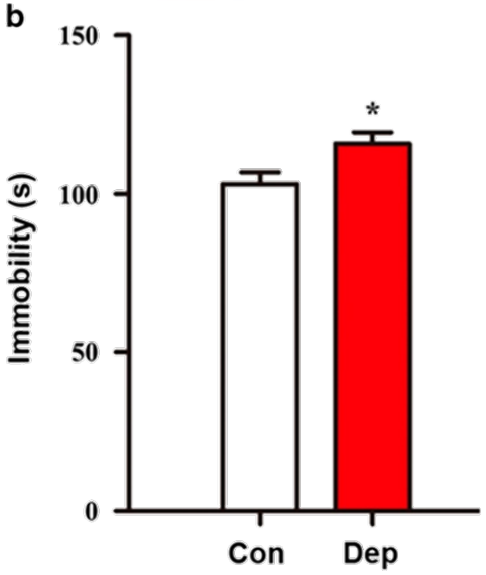


Zheng et al, Molecular Psychiatry 2016

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

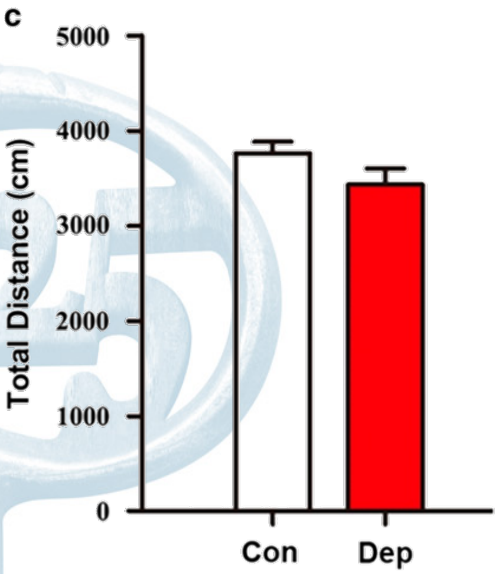
forced swim test

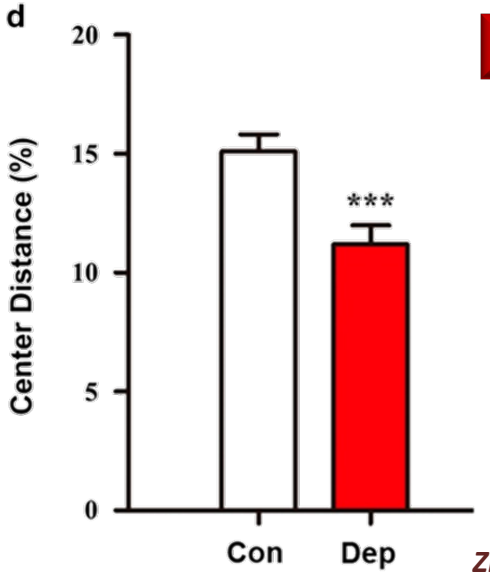




tail suspension test

overall locomotor activity

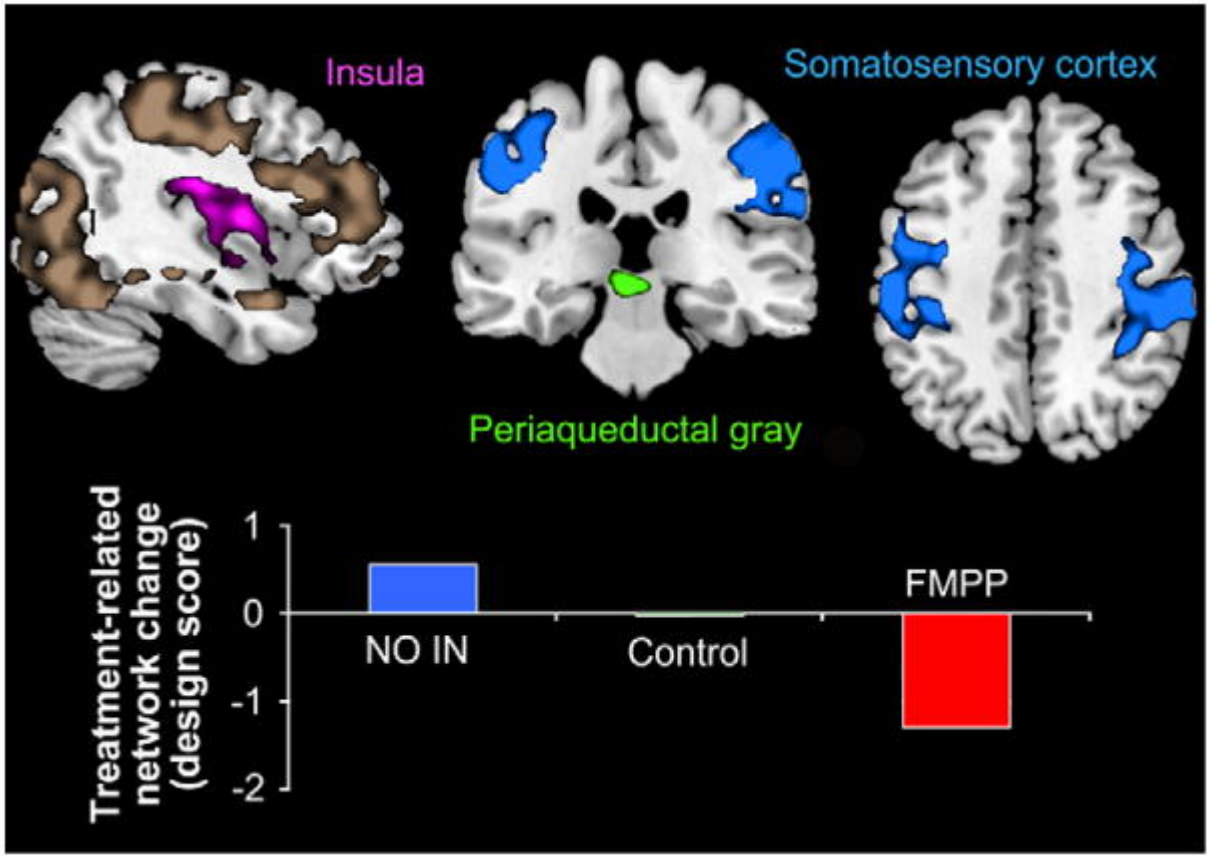




open field test

Zheng et al, Molecular Psychiatry 2016

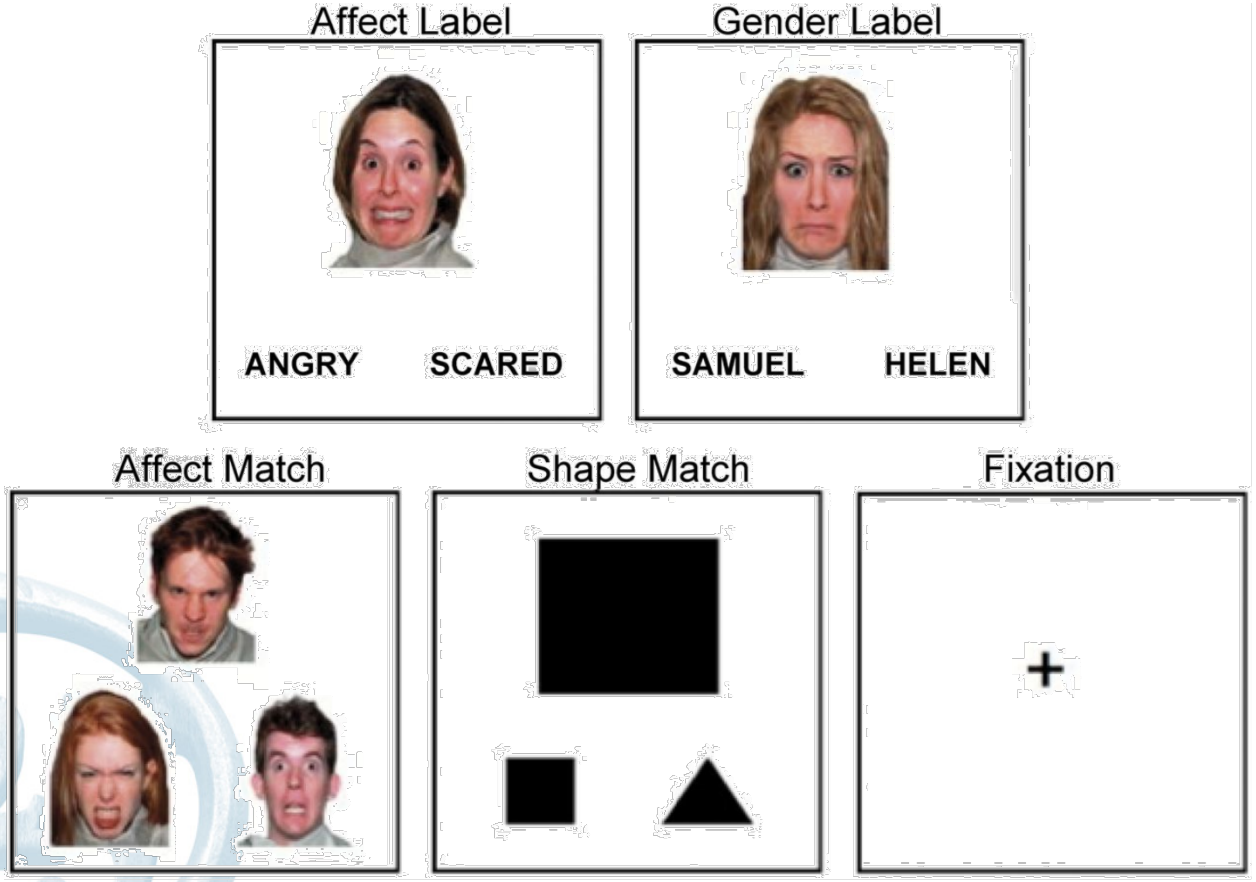
Probiotic intake alters resting state brain function in healthy humans



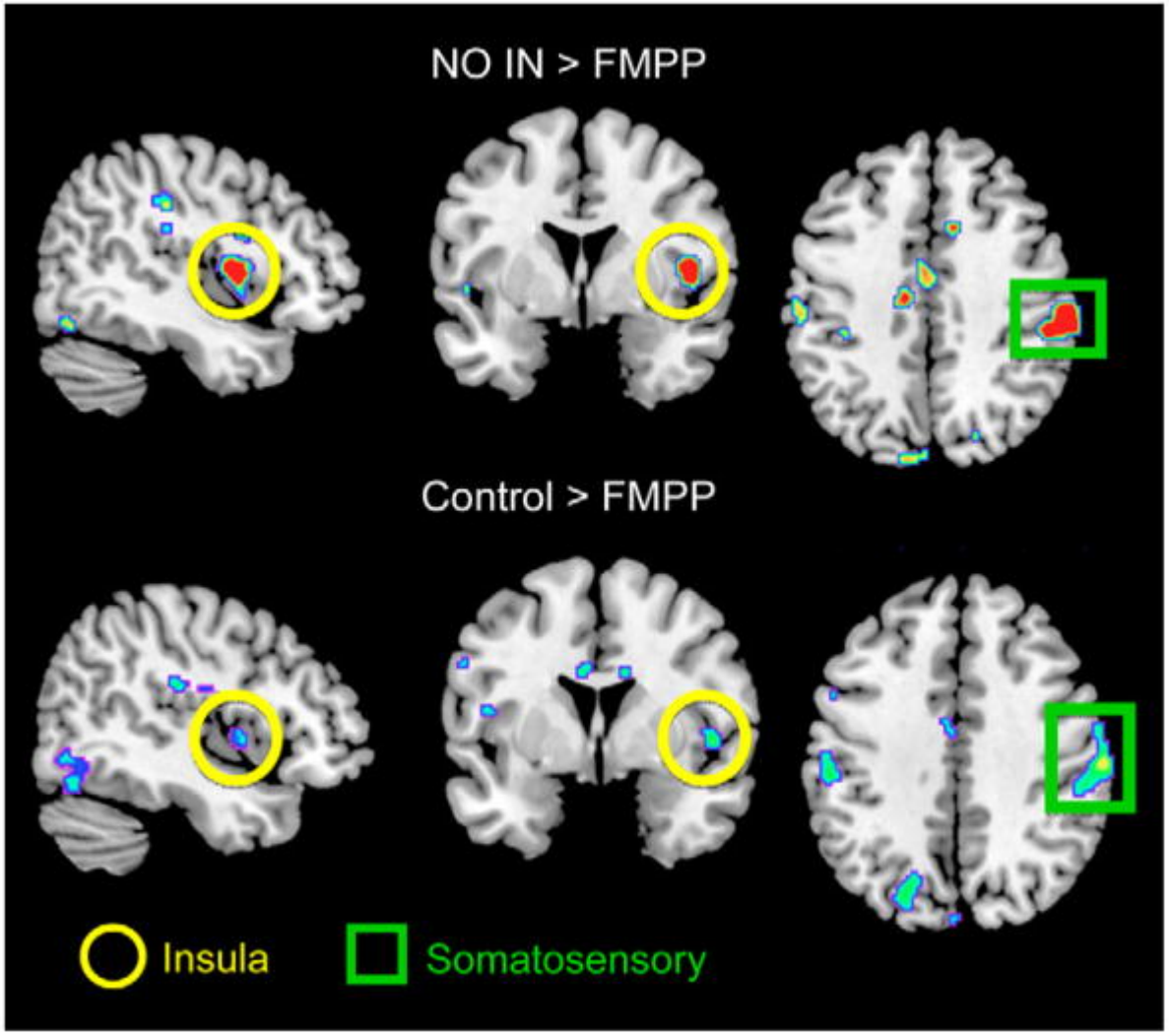


Tillisch,..., Mayer, Gastroenterology 2013

How to measure emotional brain response in humans?
Affect labeling/matching task



Probiotic intake alters brain responses to emotional attention task in healthy humans

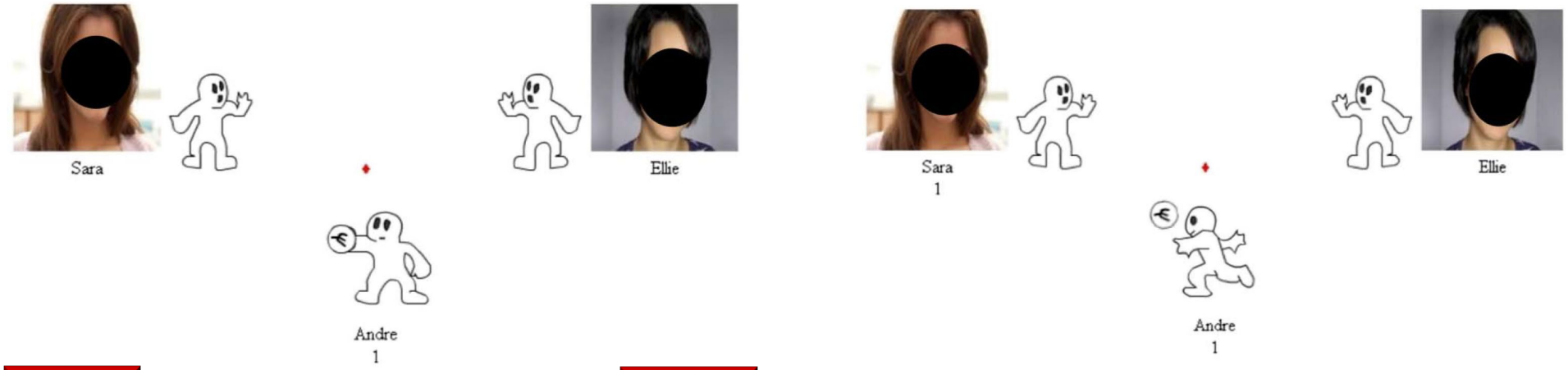




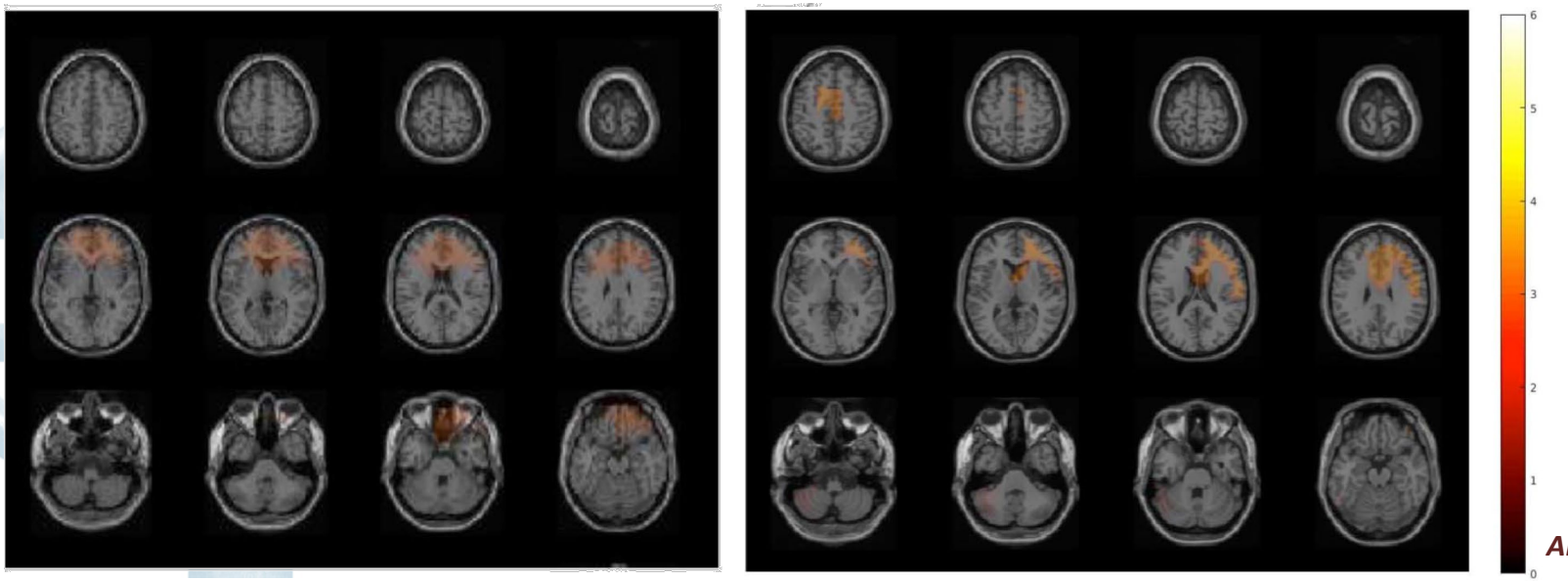
Tillisch,..., Mayer, Gastroenterology 2013

Bifidobacterium longum 1714™ Strain Modulates Brain Activity of Healthy Volunteers During Social Stress

Huiying Wang, PhD[1,2,3], Christoph Braun, PhD[2,4], Eileen F. Murphy, PhD[5] and Paul Enck, PhD[1]



rest **stress**



Wang et al

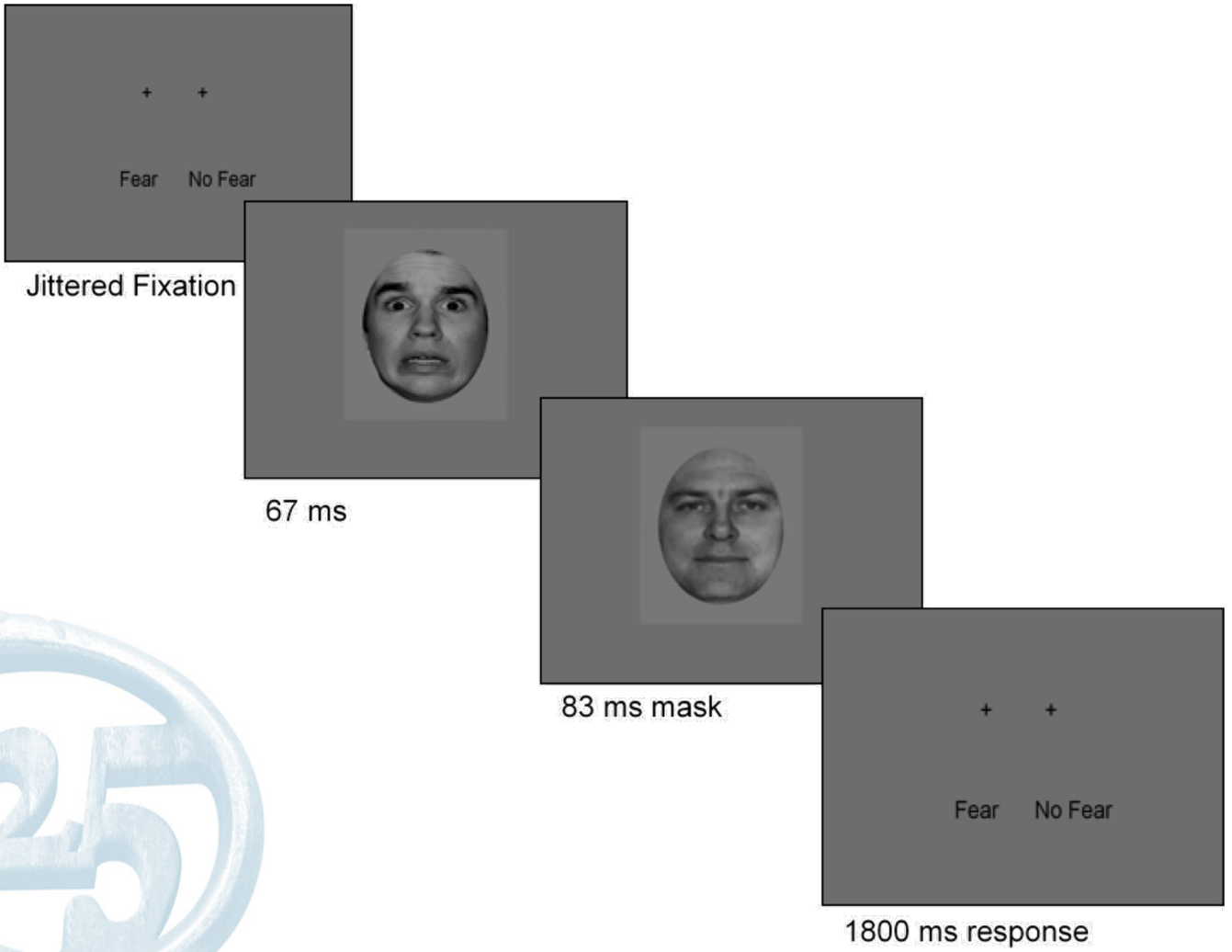
Am J Gastroenterol 2019

Bifidobacterium longum 1714 Does Not Modulate Reactivity to Social Stress

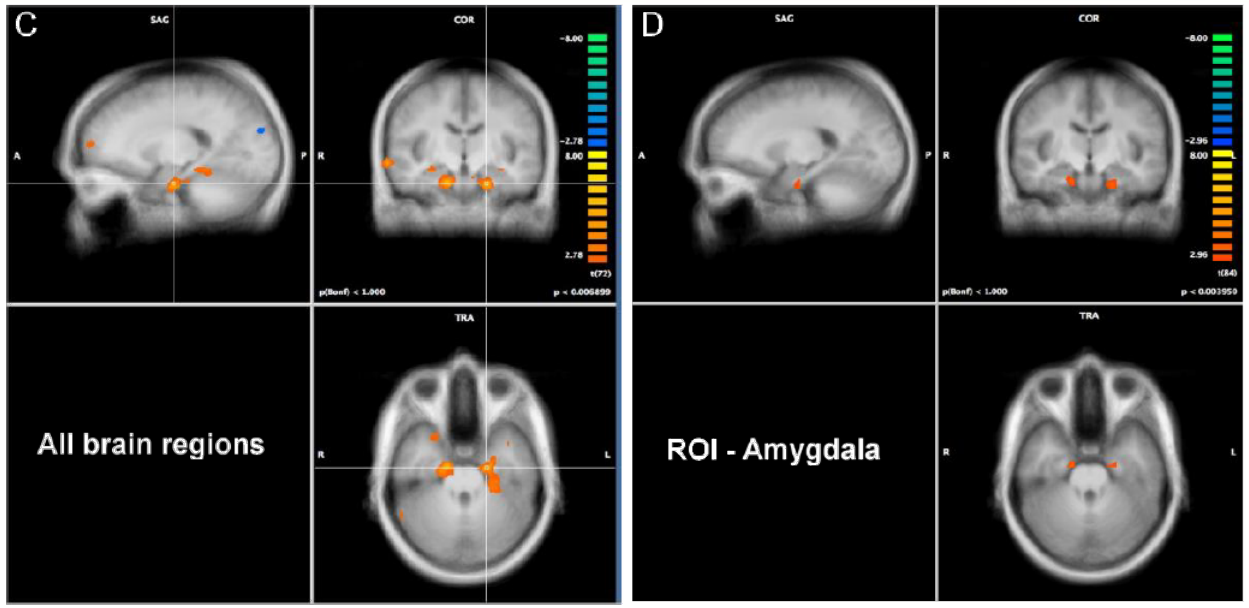
Boushra Dalile, MSc[1], Bram Vervliet, PhD[2] and Lukas Van Oudenhove, MD, PhD[1]

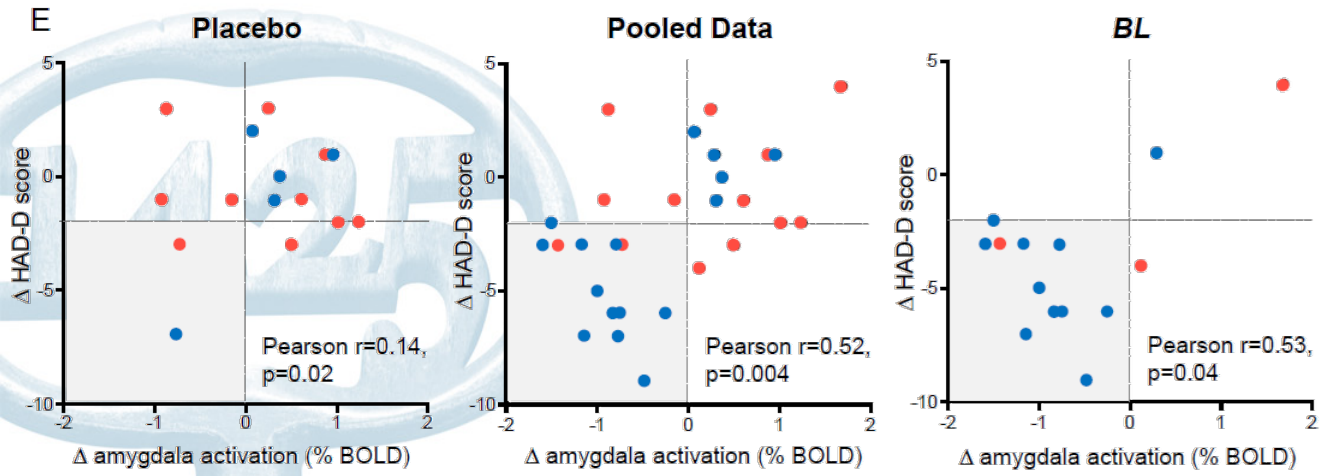
Am J Gastroenterol 2019;00:1. https://doi.org/10.14309/ajg.0000000000000377

How to measure emotional brain response in humans?
Backward masking paradigm



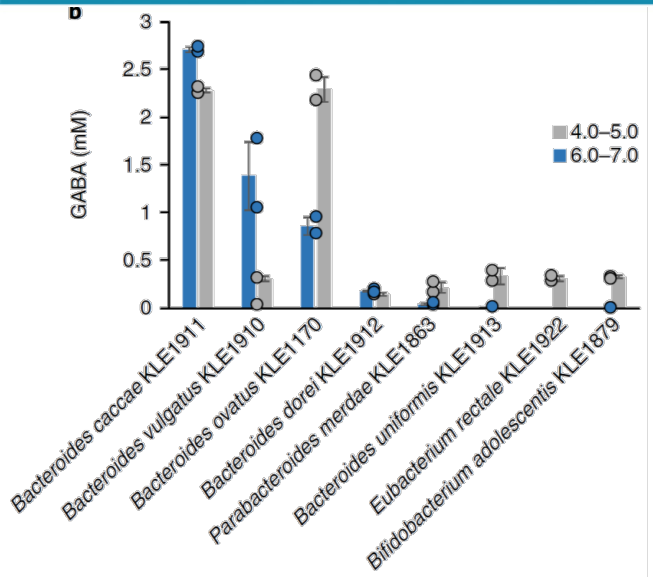
Probiotic treatment decreases depressive symptoms & emotional brain responses in IBS

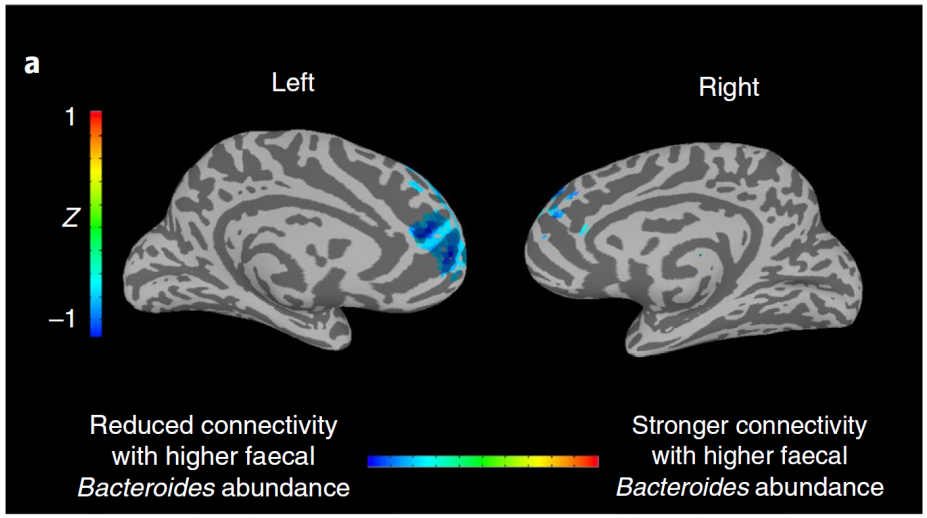


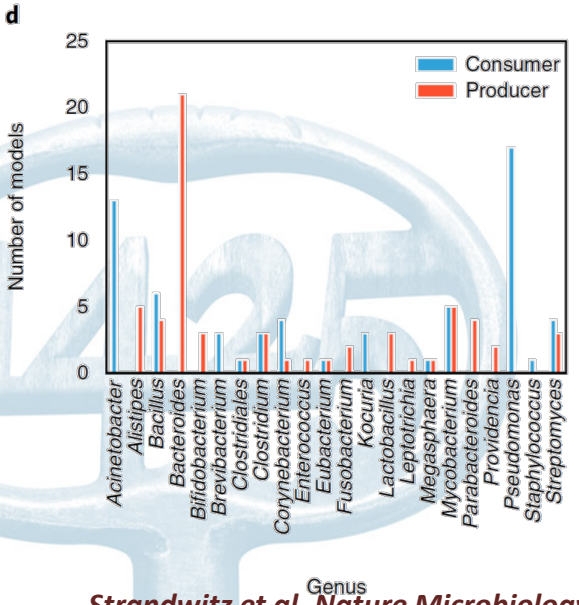


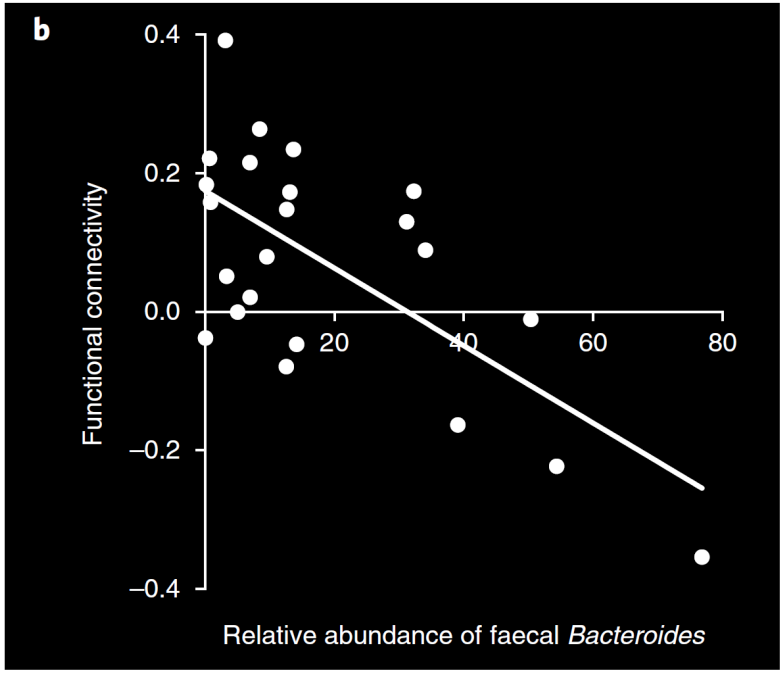
Pinto Sanchez et al, Gastroenterology 2017

Abundance of GABA-producing Bacteroides spp. with brain functional connectivity in MDD









Strandwitz et al, Nature Microbiology 2018

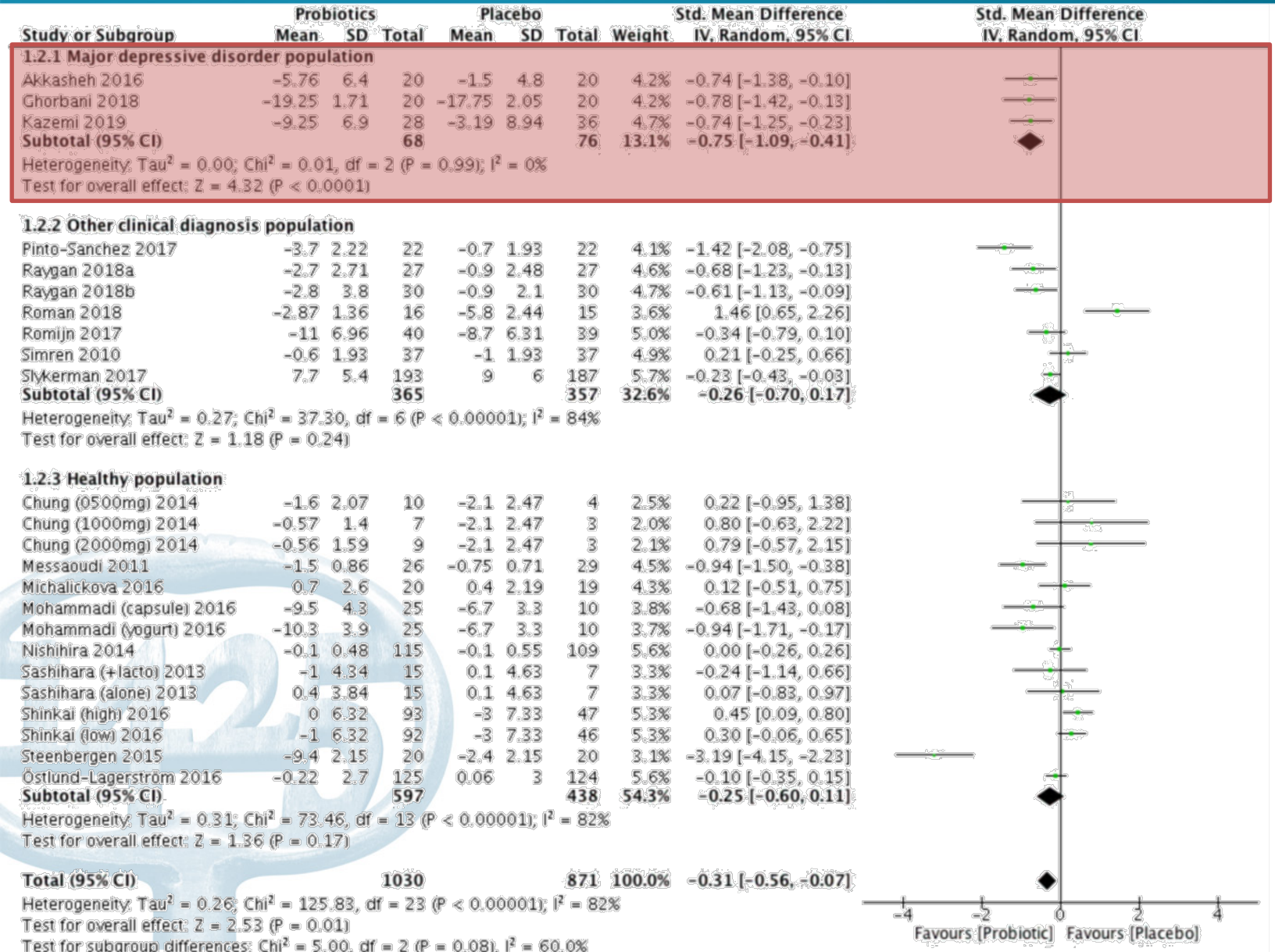
Ready for the clinic?
probiotics & depression: review of meta-analyses (!)

Table 2. GRADE evidence profiles: Quality assessments of included systematic reviews

Reference (study design)	n studies	Result	Publication bias	Quality
Ng *et al.*[32] (RCT)	12	Negative	Undetected	(+)(+)(+)(+) High
McKean *et al.*[30] (RCT)	7	Positive	Suspected	(+)( )( )( ) Very Low
Wallace *et al.*[33] (RCT)	8	-	Suspected	(+)( )( )( ) Very Low
Wang *et al.*[21] (RCT)	12	-	Undetected	(+)(+)(+)(+) High
Pirbaglou *et al.*[34] (RCT)	10	-	Suspected	(+)(+)(+)( ) Moderate
Huang *et al.*[31] (RCT)	5	Positive	Undetected	(+)(+)(+)(+) High
Romijn *et al.*[35] (RCT)	8	-	Suspected	(+)( )( )( ) Very Low

Nadeem et al, Psychiatr Clin Neurosci 2019

Ready for the clinic?
probiotics & depression: meta-analysis



Study or Subgroup	Probiotics Mean	SD	Total	Placebo Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.2.1 Major depressive disorder population								
Akkasheh 2016	-5.76	6.4	20	-1.5	4.8	20	4.2%	-0.74 [-1.38, -0.10]
Ghorbani 2018	-19.25	1.71	20	-17.75	2.05	20	4.2%	-0.78 [-1.42, -0.13]
Kazemi 2019	-9.25	6.9	28	-3.19	8.94	36	4.7%	-0.74 [-1.25, -0.23]
Subtotal (95% CI)			**68**			**76**	**13.1%**	**-0.75 [-1.09, -0.41]**
Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 2 (P = 0.99); I² = 0%								
Test for overall effect: Z = 4.32 (P < 0.0001)								
1.2.2 Other clinical diagnosis population								
Pinto-Sanchez 2017	-3.7	2.22	22	-0.7	1.93	22	4.1%	-1.42 [-2.08, -0.75]
Raygan 2018a	-2.7	2.71	27	-0.9	2.48	27	4.6%	-0.68 [-1.23, -0.13]
Raygan 2018b	-2.8	3.8	30	-0.9	2.1	30	4.7%	-0.61 [-1.13, -0.09]
Roman 2018	-2.87	1.36	16	-5.8	2.44	15	3.6%	1.46 [0.65, 2.26]
Romijn 2017	-11	6.96	40	-8.7	6.31	39	5.0%	-0.34 [-0.79, 0.10]
Simren 2010	-0.6	1.93	37	-1	1.93	37	4.9%	0.21 [-0.25, 0.66]
Slykerman 2017	7.7	5.4	193	9	6	187	5.7%	-0.23 [-0.43, -0.03]
Subtotal (95% CI)			**365**			**357**	**32.6%**	**-0.26 [-0.70, 0.17]**
Heterogeneity: Tau² = 0.27; Chi² = 37.30, df = 6 (P < 0.00001); I² = 84%								
Test for overall effect: Z = 1.18 (P = 0.24)								
1.2.3 Healthy population								
Chung (0500mg) 2014	-1.6	2.07	10	-2.1	2.47	4	2.5%	0.22 [-0.95, 1.38]
Chung (1000mg) 2014	-0.57	1.4	7	-2.1	2.47	3	2.0%	0.80 [-0.63, 2.22]
Chung (2000mg) 2014	-0.56	1.59	9	-2.1	2.47	3	2.1%	0.79 [-0.57, 2.15]
Messaoudi 2011	-1.5	0.86	26	-0.75	0.71	29	4.5%	-0.94 [-1.50, -0.38]
Michalickova 2016	0.7	2.6	20	0.4	2.19	19	4.3%	0.12 [-0.51, 0.75]
Mohammadi (capsule) 2016	-9.5	4.3	25	-6.7	3.3	10	3.8%	-0.68 [-1.43, 0.08]
Mohammadi (yogurt) 2016	-10.3	3.9	25	-6.7	3.3	10	3.7%	-0.94 [-1.71, -0.17]
Nishihira 2014	-0.1	0.48	115	-0.1	0.55	109	5.6%	0.00 [-0.26, 0.26]
Sashihara (+lacto) 2013	-1	4.34	15	0.1	4.63	7	3.3%	-0.24 [-1.14, 0.66]
Sashihara (alone) 2013	0.4	3.84	15	0.1	4.63	7	3.3%	0.07 [-0.83, 0.97]
Shinkai (high) 2016	0	6.32	93	-3	7.33	47	5.3%	0.45 [0.09, 0.80]
Shinkai (low) 2016	-1	6.32	92	-3	7.33	46	5.3%	0.30 [-0.06, 0.65]
Steenbergen 2015	-9.4	2.15	20	-2.4	2.15	20	3.1%	-3.19 [-4.15, -2.23]
Östlund-Lagerström 2016	-0.22	2.7	125	0.06	3	124	5.6%	-0.10 [-0.35, 0.15]
Subtotal (95% CI)			**597**			**438**	**54.3%**	**-0.25 [-0.60, 0.11]**
Heterogeneity: Tau² = 0.31; Chi² = 73.46, df = 13 (P < 0.00001); I² = 82%								
Test for overall effect: Z = 1.36 (P = 0.17)								
Total (95% CI)			**1030**			**871**	**100.0%**	**-0.31 [-0.56, -0.07]**
Heterogeneity: Tau² = 0.26; Chi² = 125.83, df = 23 (P < 0.00001); I² = 82%								
Test for overall effect: Z = 2.53 (P = 0.01)								
Test for subgroup differences: Chi² = 5.00, df = 2 (P = 0.08), I² = 60.0%								

Std. Mean Difference IV, Random, 95% CI

-4 -2 0 2 4

Favours [Probiotic] Favours [Placebo]

Goh et al, Psychiatry Res 2019

Behavioral and Brain Sciences

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Microbiota-gut-brain research: A critical analysis

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Target Article

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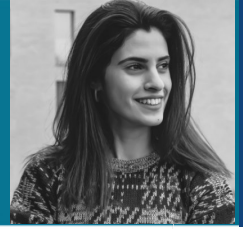
Hooks et al, Behav Brain Sci 2019
Dalile et al, Behav Brain Sci 2019

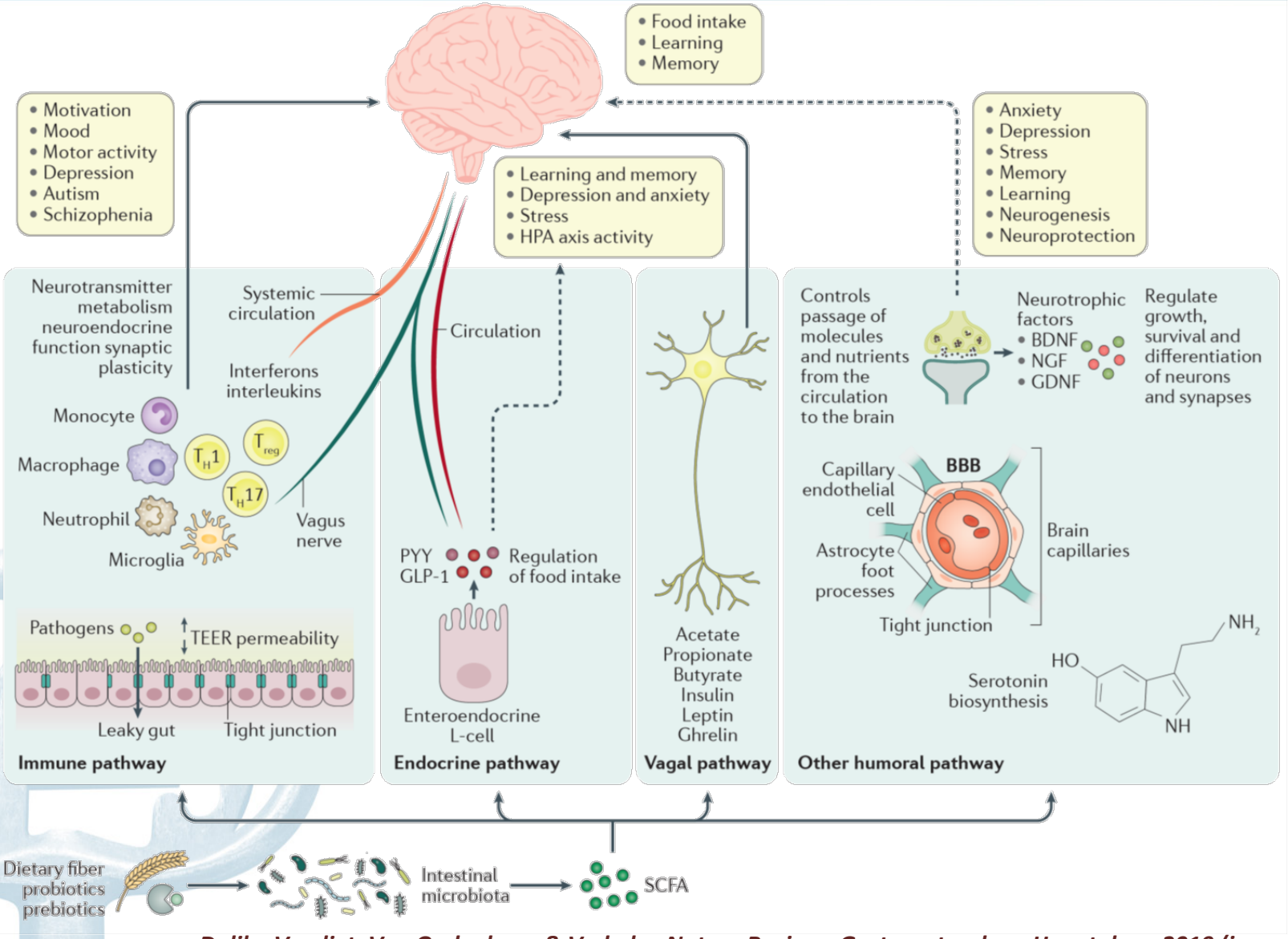
Microbiota-gut-brain axis research
pitfalls

- **microbiota methodology**
  - complex field of its own!
  - what is a “normal” microbiota? what is “dysbiosis”?
  - germ-free animals
- **neuroendocrine & behavioural tests and models**
  - translation rodents – humans
- **statistical methodology**
  - study designs/paradigms
  - multiple testing issues!
- **strong claims & interpretations**
  - causality – “cum hoc non est propter hoc”
  - evolutionary claims
  - probiotic claims
  - science communication

Hooks et al, Behav Brain Sci 2019

From associations to mechanisms
short chain fatty acids

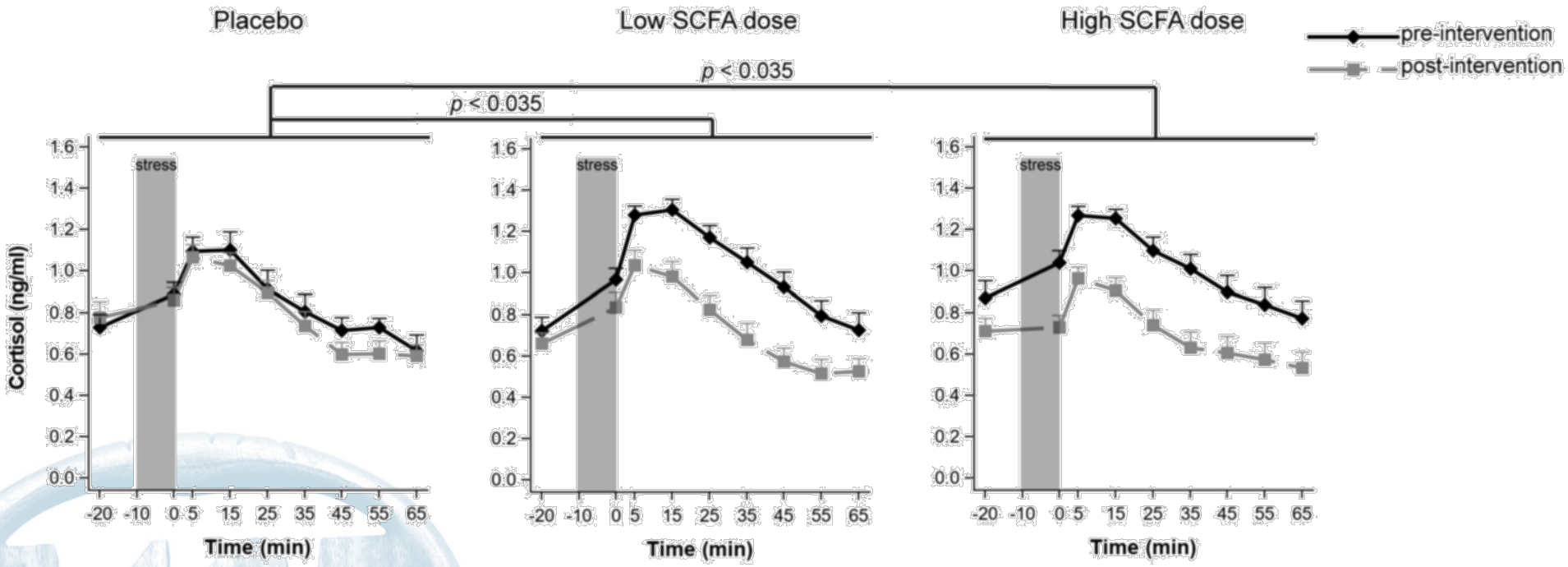




Dalile, Vervliet, Van Oudenhove & Verbeke, Nature Reviews Gastroenterology Hepatology 2019 (in press)

Colon-delivered short-chain fatty acids attenuate the cortisol response to stress

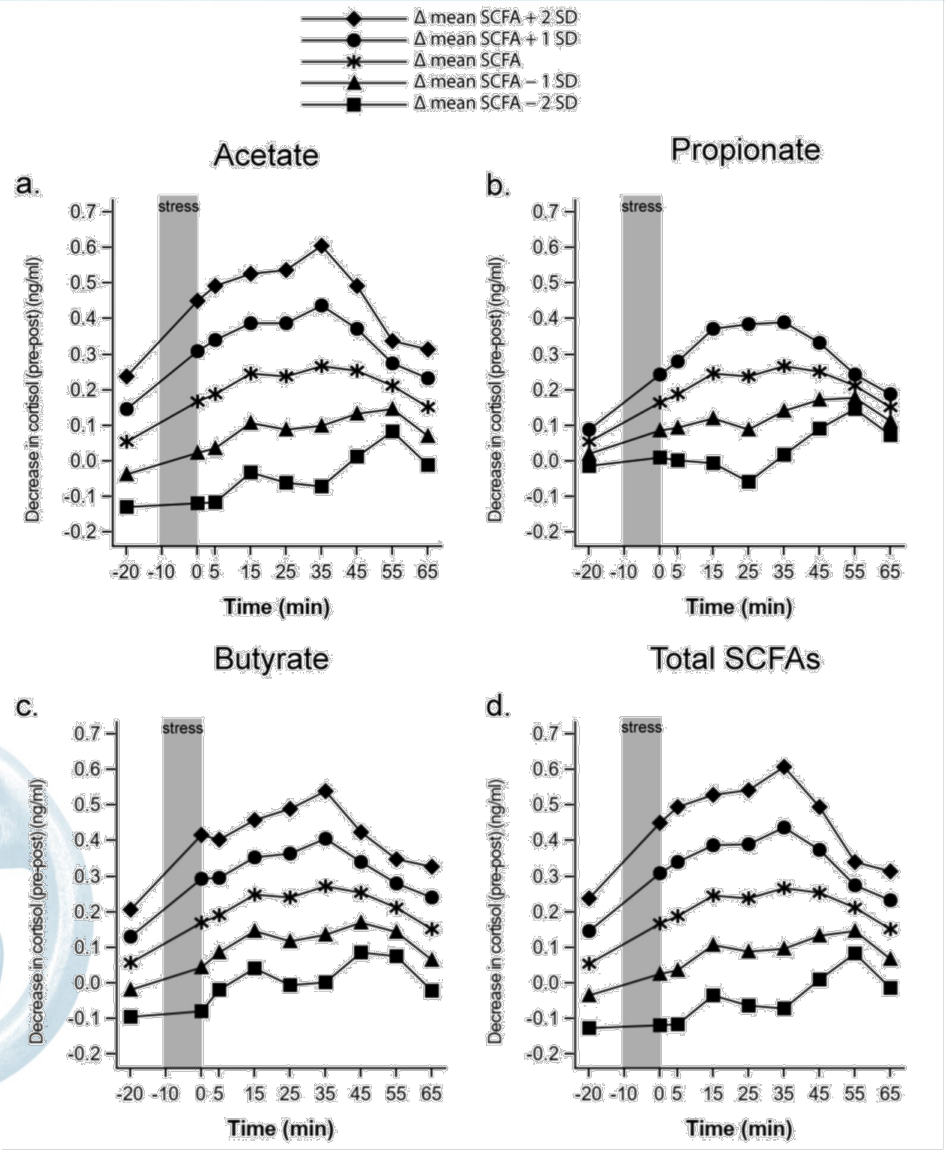




Dalile, Vervliet, Bergonzelli, Verbeke & Van Oudenhove, Neuropsycopharmacology 2020

Colon-delivered short-chain fatty acids attenuate the cortisol response to stress





Dalile, Vervliet, Bergonzelli, Verbeke & Van Oudenhove, Neuropsycopharmacology 2020

Take home messages

- The **gut microbiota** have recently been identified as a key **new player** in **bidirectional gut-brain communication**
- Gut microbiota composition & function can be **altered** by **pro- and prebiotics**, and **fecal microbiota transfer**
- **Microbiota** targeted interventions impact on **psychological and brain function** in rodents
- **Human translation** is lagging behind, and more **mechanistic and clinical research** is needed before microbiota targeted interventions are ready for the **(psychiatric) clinic**