CP-konferansen
Oslo 18th March 2019

Karolinska Institutet

Microbiota
Har tarmfloran en rolle i hjernens utveckling?

Hans Forssberg, MD, PhD
Neuropaediatrics
Karolinska Institutet

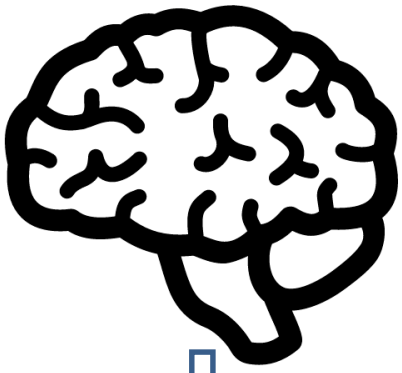
Human Brain Development

Genes

Epigenetics

Environment

Brain



Chromosomal anomalies
Copy number variants
Single polymorphisms

Intra uterine conditions
- Maternal stress, smoking, medication

Preterm birth

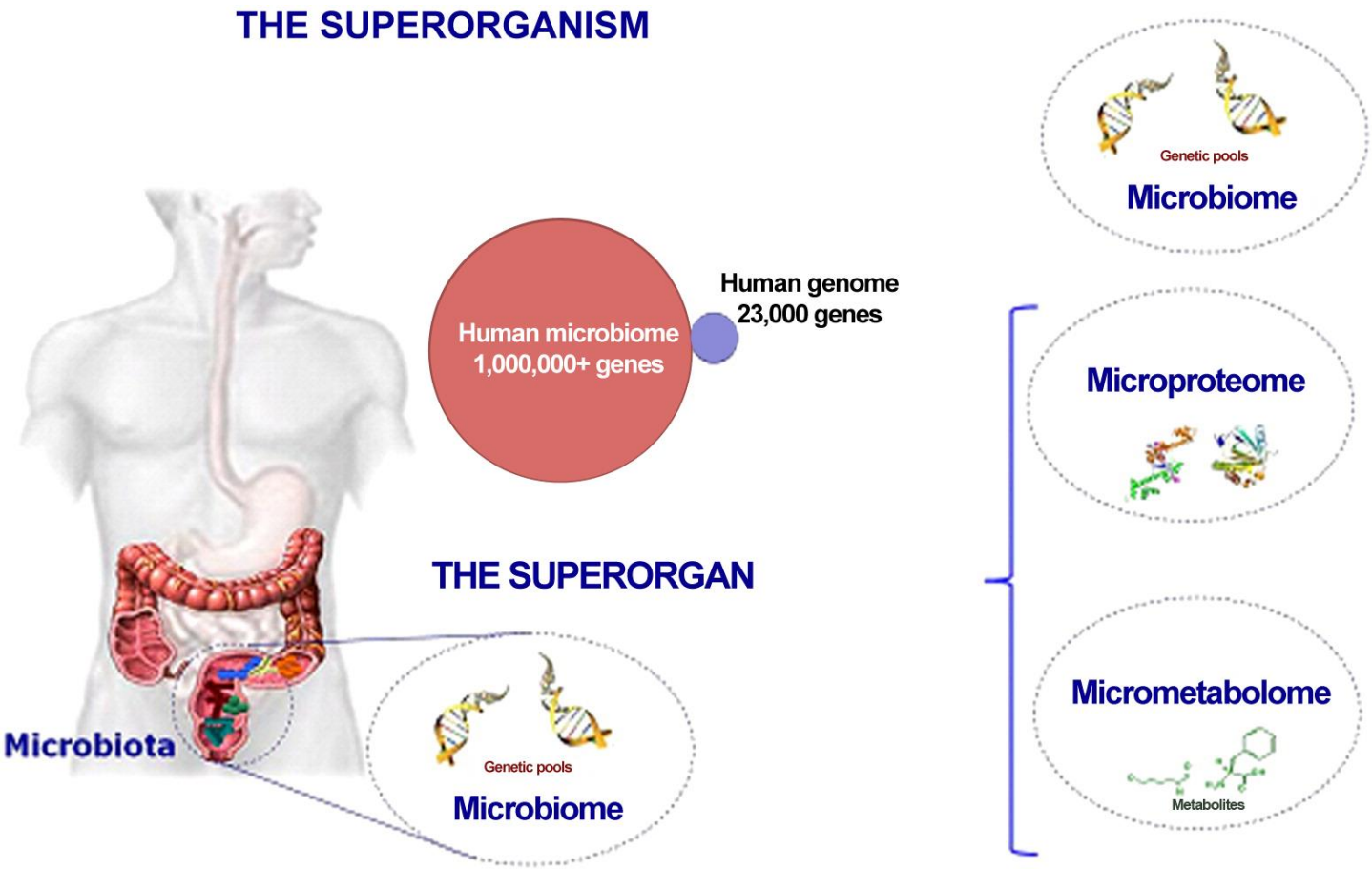
Postnatal & Childhood
- Nutrition, unsafe conditions, stimulating environment, parent interaction

Phenotype

Neurodevelopmental disorders:
ADHD, ASD,ID,DCD, SLI, Dyslexia,Tourette, OBD

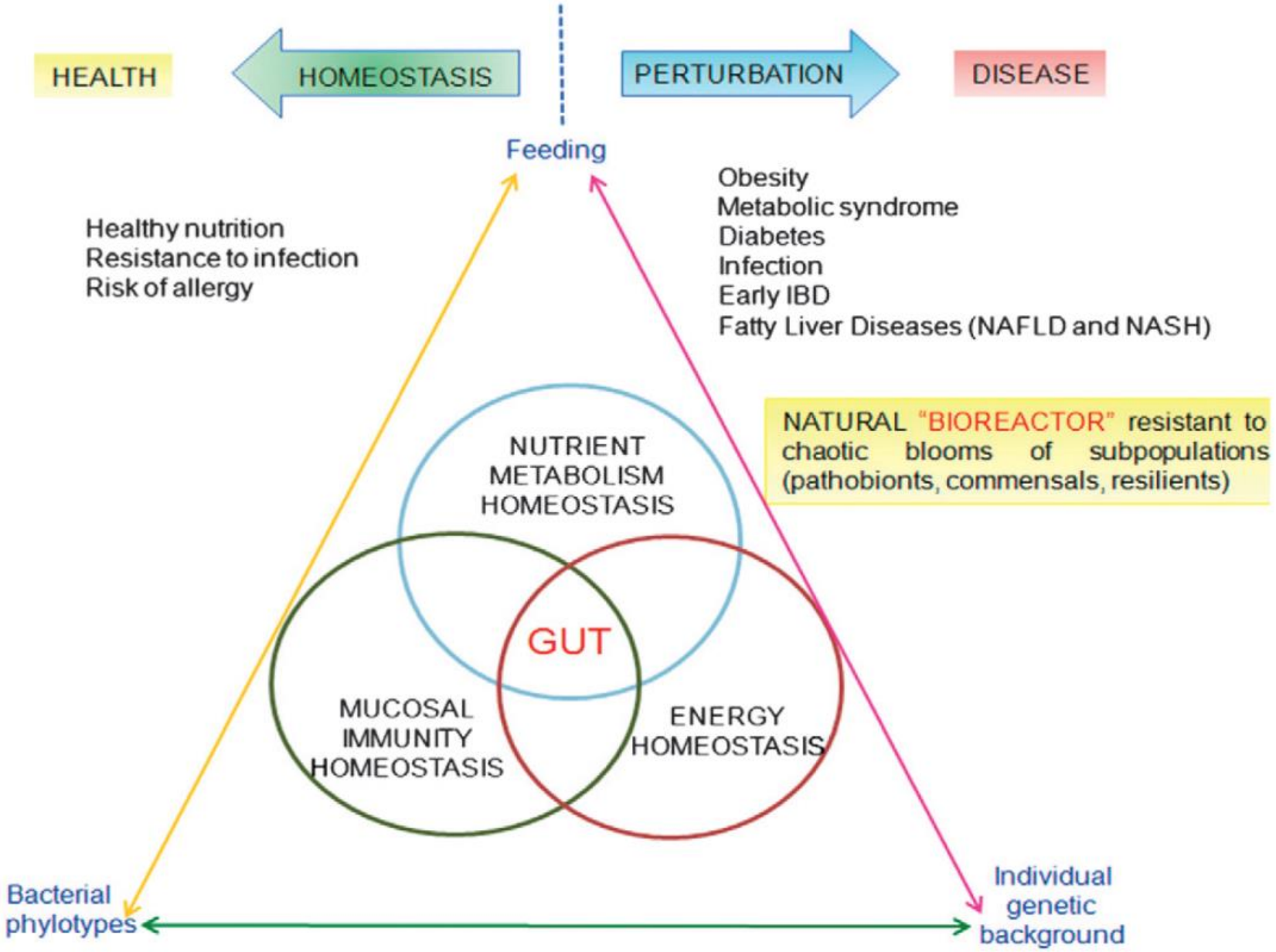
Microbiome

THE MICROBIOME



Del Chierico F et al 2012

THE MICROBIOME



Del Chierico F et al 2012

The LANCET series on ECD

Series

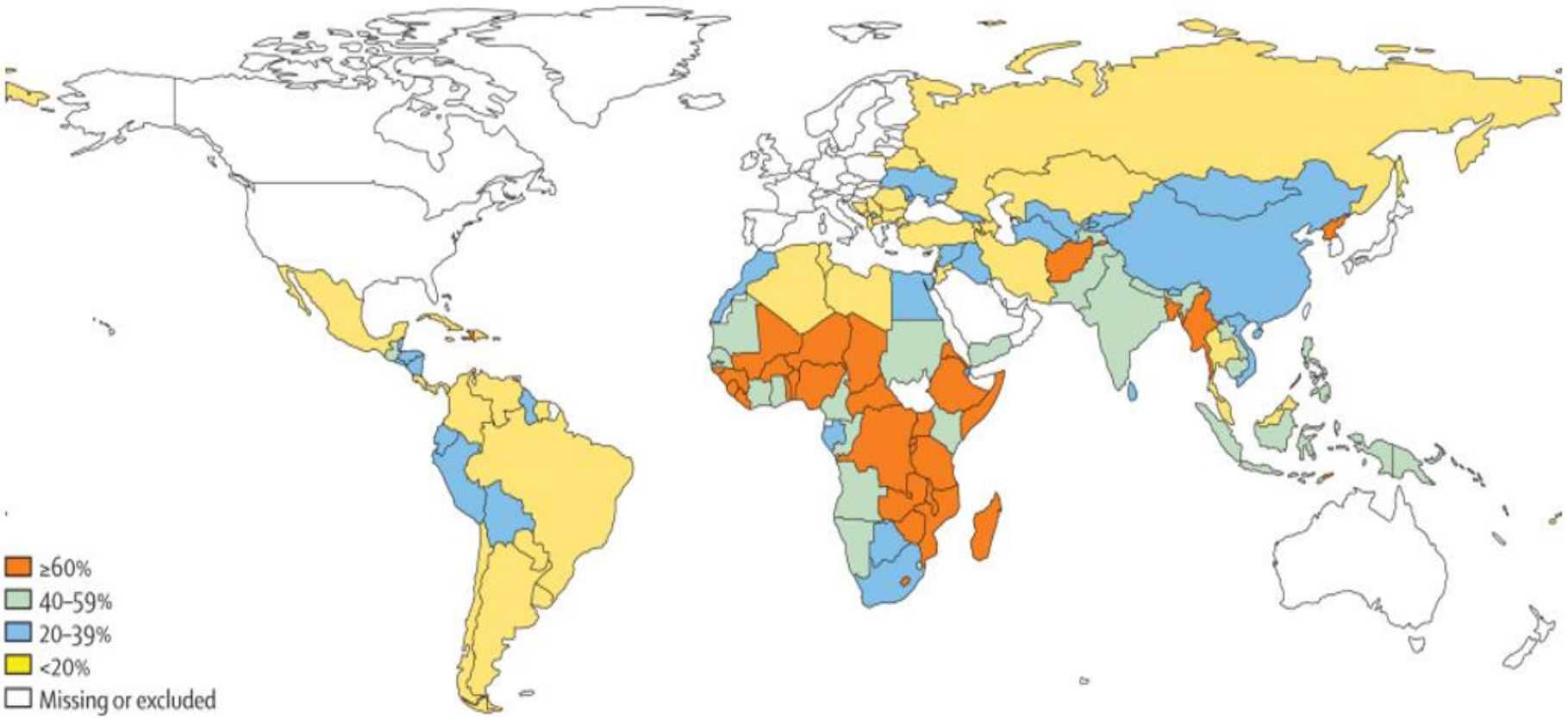
www.thelancet.com Published Online October 4, 2016 http://dx.doi.org/10.1016/S0140-6736(16)31698-1

Advancing Early Childhood Development: from Science to Scale 1

Early childhood development coming of age: science through the life course

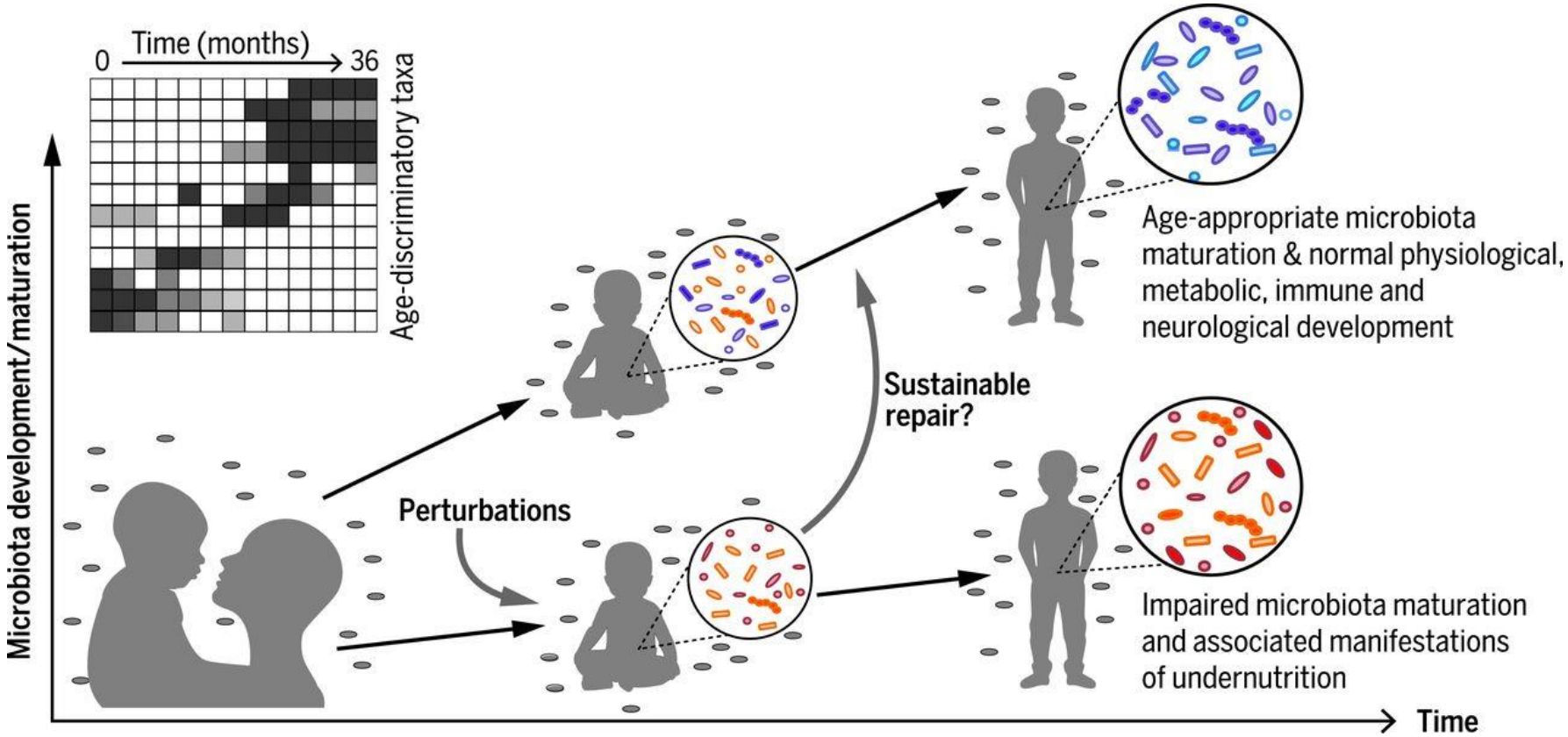
Estimates, based on proxy measures of stunting and poverty, indicate that 250 million children (43%) younger than 5 years in low-income and middle-income countries are at risk of not reaching their developmental potential.

Global percentage of children younger than 5 years at risk of poor development 2010



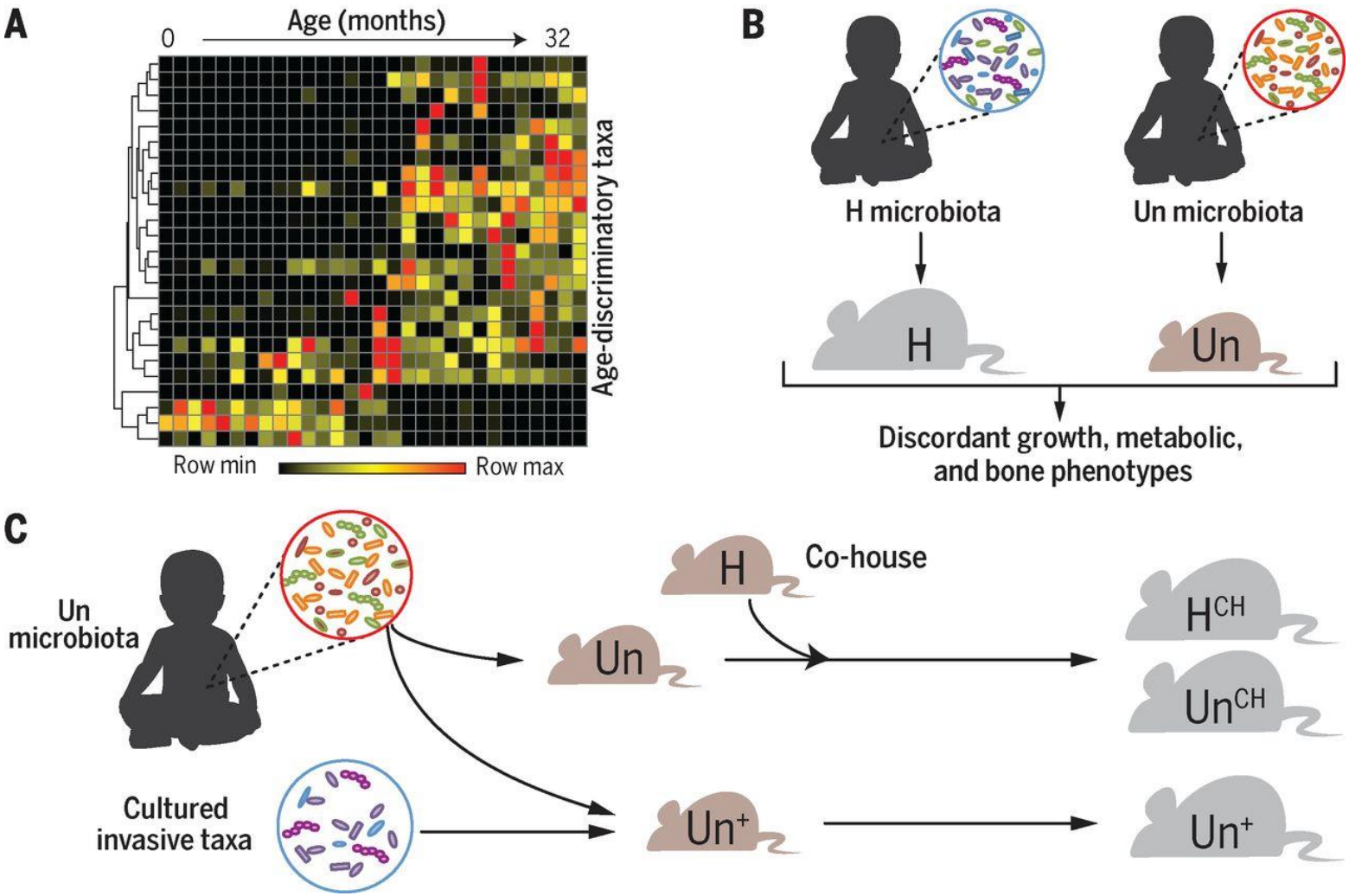
Chunling Lu, Maureen M Black, Linda M Richter, The Lancet Global Health, 2016,

The concept that impaired postnatal gut microbiota development (maturation) is causally related to childhood undernutrition.



Blanton et al. Science 2016

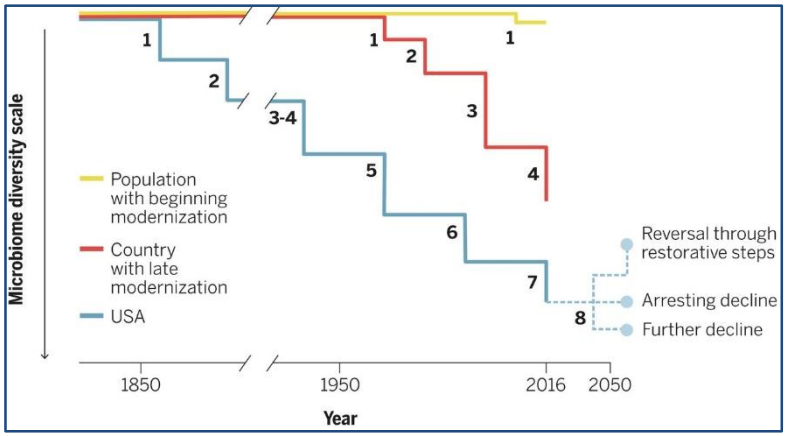
Preclinical evidence that gut microbiota immaturity is causally related to childhood undernutrition



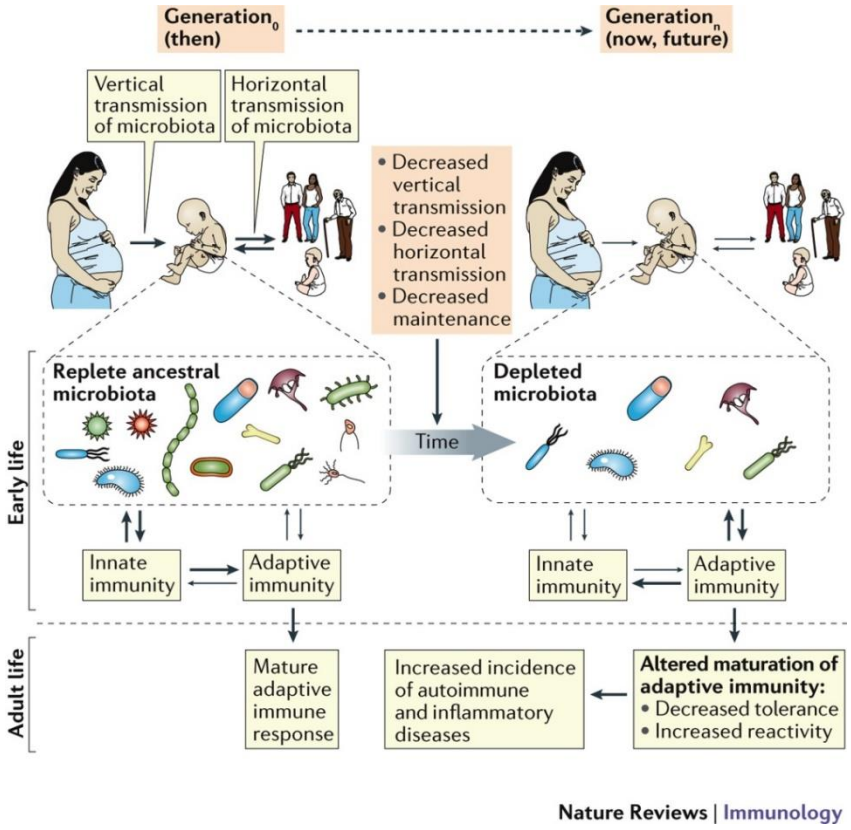
Blanton et al. Science 2016

The theory of disappearing microbiota

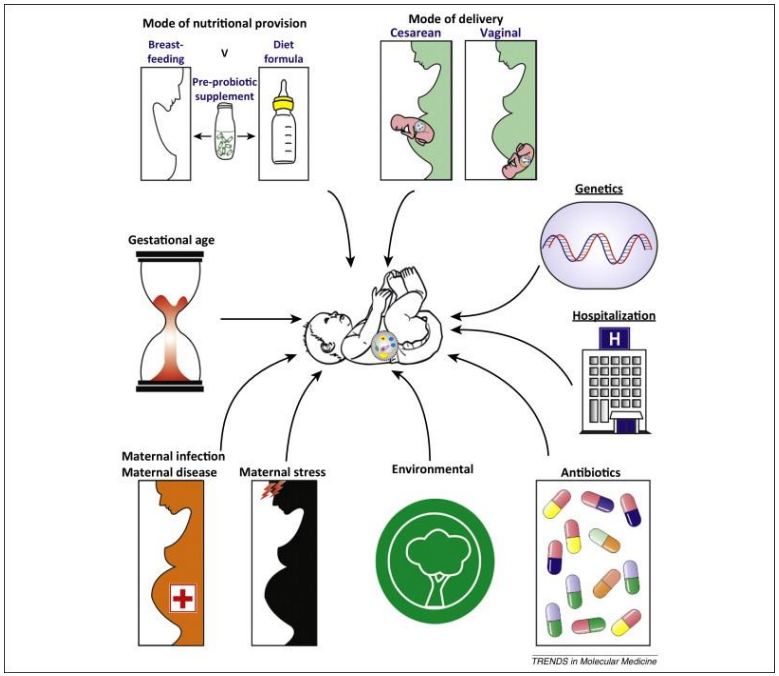
Increasing incidence of:
obesity, asthma, hay fever, juvenile diabetes, inflammatory bowel disease and autism spectrum?



Blaser: Science 2016



Blaser, Dominguez-Bello 2016



Borre, O'Keeffe, Clarke, Stanton, Dinan, Cryan: J Mol Med 2014

ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803-5253

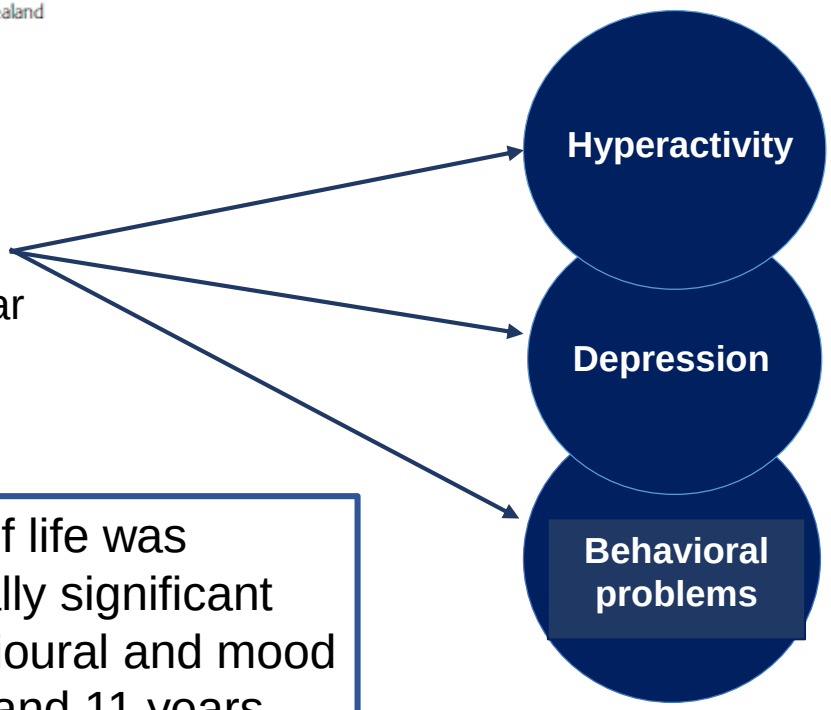
REGULAR ARTICLE

Antibiotics in the first year of life and subsequent neurocognitive outcomes

Rebecca F. Slykerman (r.slykerman@auckland.ac.nz)[1], John Thompson[1], Karen E. Waldie[2], Rinki Murphy[3], Clare Wall[4], Edwin A. Mitchell[1]

1.Department of Paediatrics: Child and Youth Health, University of Auckland, Auckland, New Zealand
2.School of Psychology, University of Auckland, Auckland, New Zealand
3.Department of Medicine, University of Auckland, Auckland, New Zealand
4.Department of Nutrition, University of Auckland, Auckland, New Zealand

871 children
27% mother received AB
70% received AB first year
92% received AB 1- 3½ year



Antibiotic use in the first year of life was associated with small statistically significant differences in cognitive, behavioural and mood measures in children at 3.5, 7 and 11 years.

J Physiol 558.1 (2004) pp 263–275

Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice

Nobuyuki Sudo[1,2], Yoichi Chida[1], Yuji Aiba[3,4], Junko Sonoda[1], Naomi Oyama[1], Xiao-Nian Y
Chiharu Kubo[1] and Yasuhiro Koga[3]

PNAS | February 15, 2011 | vol. 108 | no. 7 | 3047–3052

Normal gut microbiota modulates brain development and behavior

Rochellys Diaz Heijtz[a,b,1], Shugui Wang[c], Farhana Anuar[d], Yu Qian[a,b], Britta Björkholm[d], Annika Samuelsson[d], Martin L. Hibberd[c], Hans Forssberg[b,e], and Sven Pettersson[c,d,1]

Neurogastroenterology & Motility
Neurogastroenterol Motil (2011) 23, 255–e119 doi: 10.1111/j.1365-2982.2010.01620.x

Reduced anxiety-like behavior and central neurochemical change in germ-free mice

K. M. NEUFELD,*,† N. KANG,*,‡ J. BIENENSTOCK*,§ & J. A. FOSTER*,‡

GASTROENTEROLOGY 2011;141:599–609

The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice

PREMYSL BERCIK,* EMMANUEL DENOU,* JOSH COLLINS,* WENDY JACKSON,* JUN LU,* JENNIFER JURY,* YIKANG DENG,* PATRICIA BLENNERHASSETT,* JOSEPH MACRI,‡ KATHY D. McCoy,* ELENA F. VERDU,* and STEPHEN M. COLLINS*

MICROBIAL ECOLOGY in Health and Disease

ORIGINAL ARTICLE

Host microbiota modulates development of social preference in mice

Tim Arentsen[1], Henrike Raith[1], Yu Qian[1], Hans Forssberg[2] and Rochellys Diaz Heijtz[1]*

[1]Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; [2]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

Molecular Psychiatry (2013) 18, 666–673

www.nature.com/mp

ORIGINAL ARTICLE

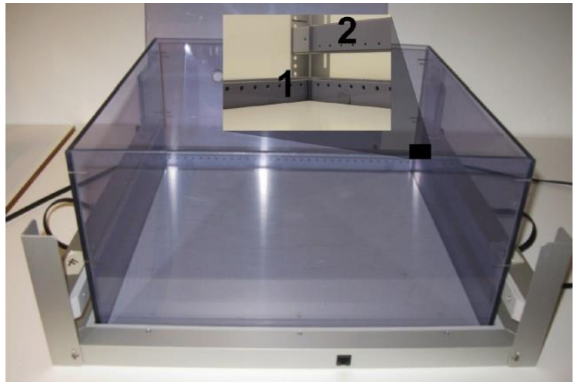
The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner

G Clarke[1,2], S Grenham[1], P Scully[1], P Fitzgerald[1], RD Moloney[1], F Shanahan[1,3], TG Dinan[1,2] and JF Cryan[1,4]

Germ-free (GF) mice are born and raised under strict sterile conditions

Battery of behavioural tests

Motor activity



Open field test

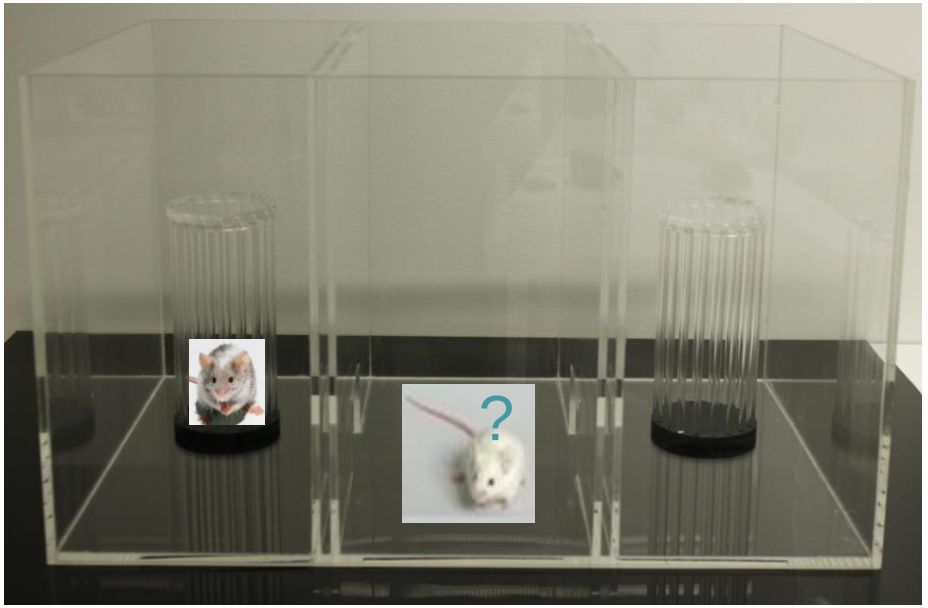
Anxiety

Elevated plus maze

Light-dark box test

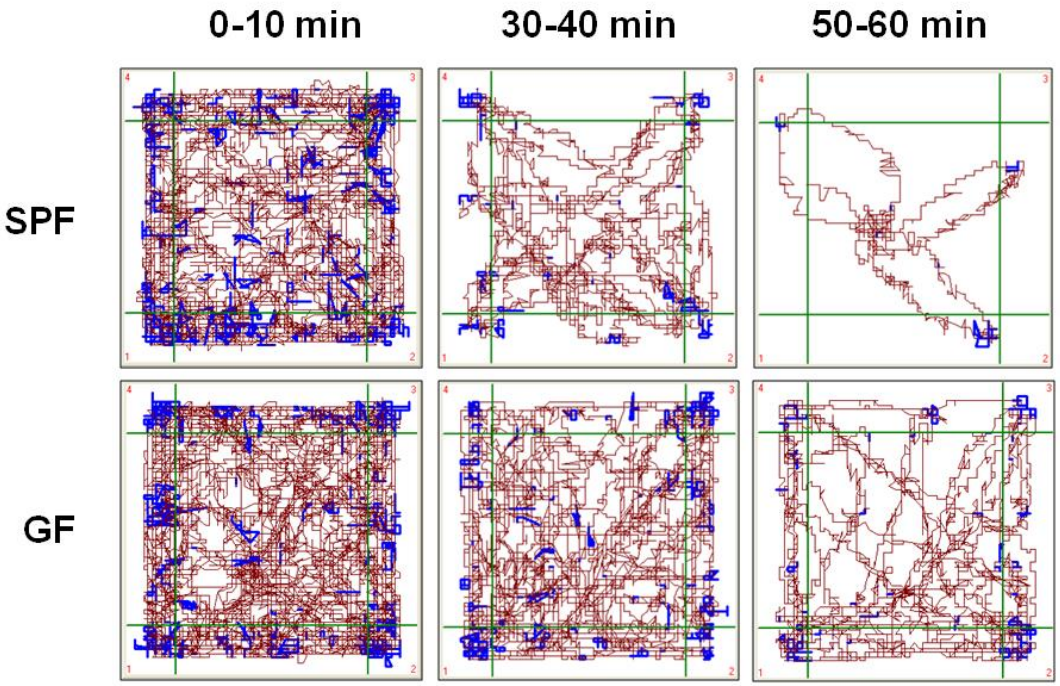
Social interaction

Three-chambered social approach task

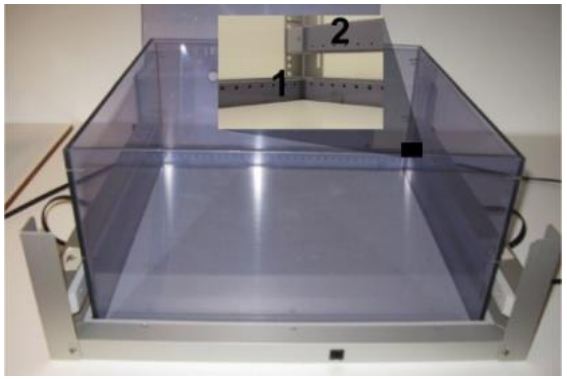


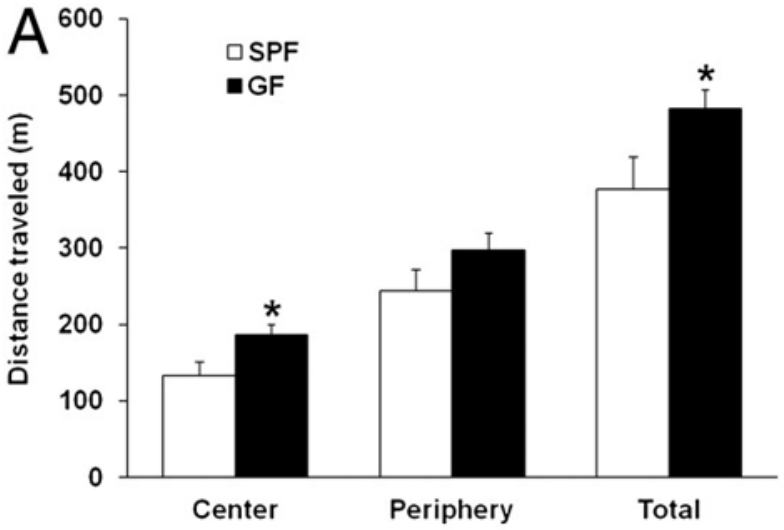
Stimulus mouse **Centre** **Novel object**

GF mice display increased spontaneous motor activity



Representative track of movements

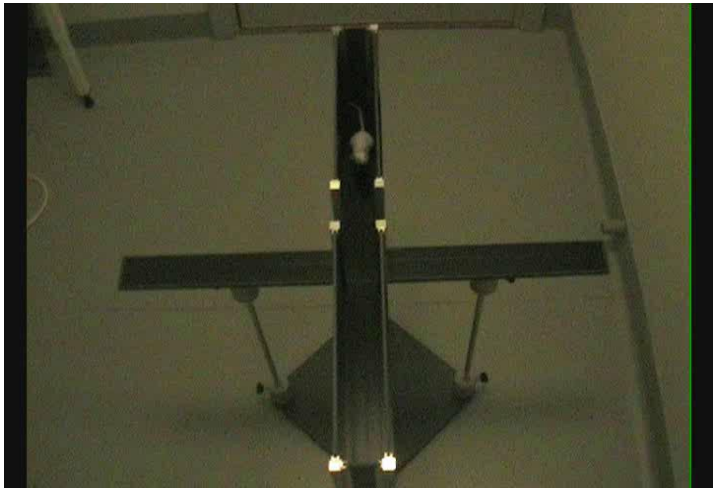




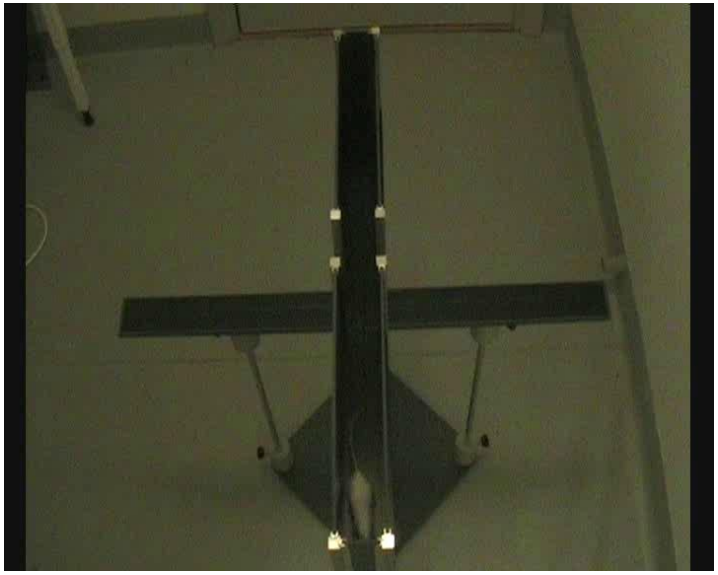
Heijtz et al. PNAS (2011)

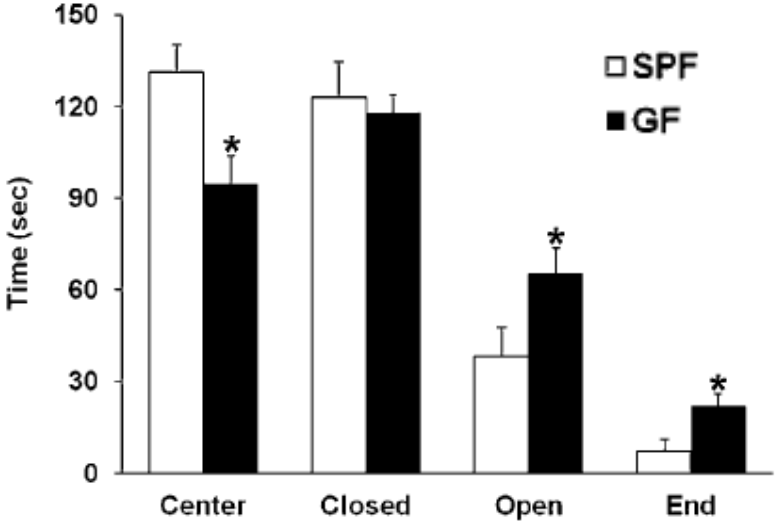
GF mice display reduced anxiety-like behaviour in the Elevated Plus Maze

SPF



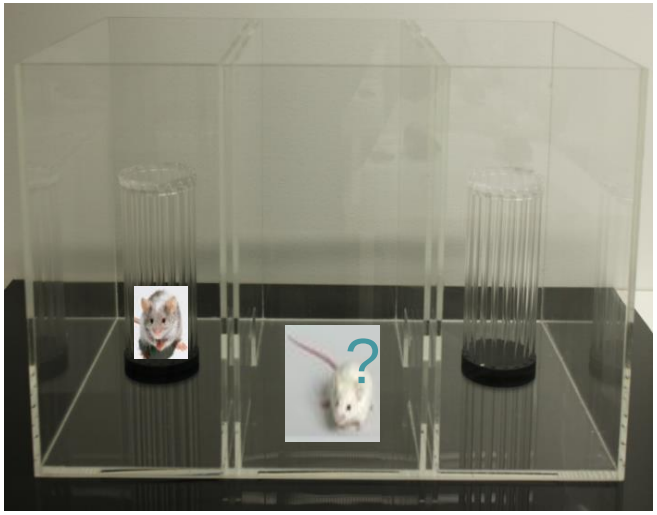
GF



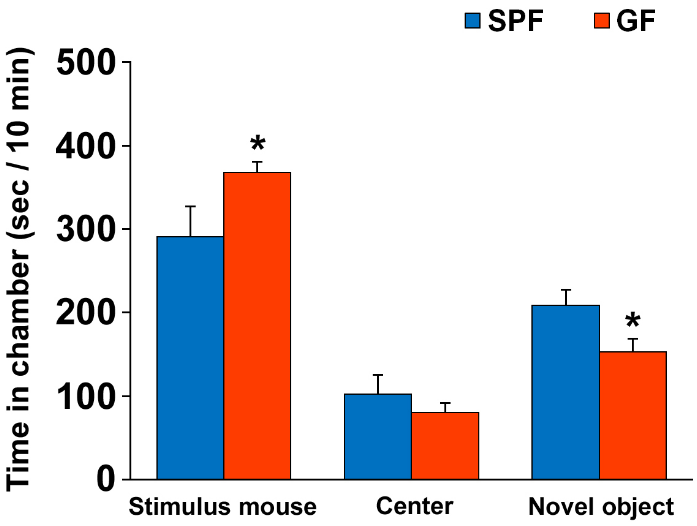


Heijtz et al. PNAS (2011)

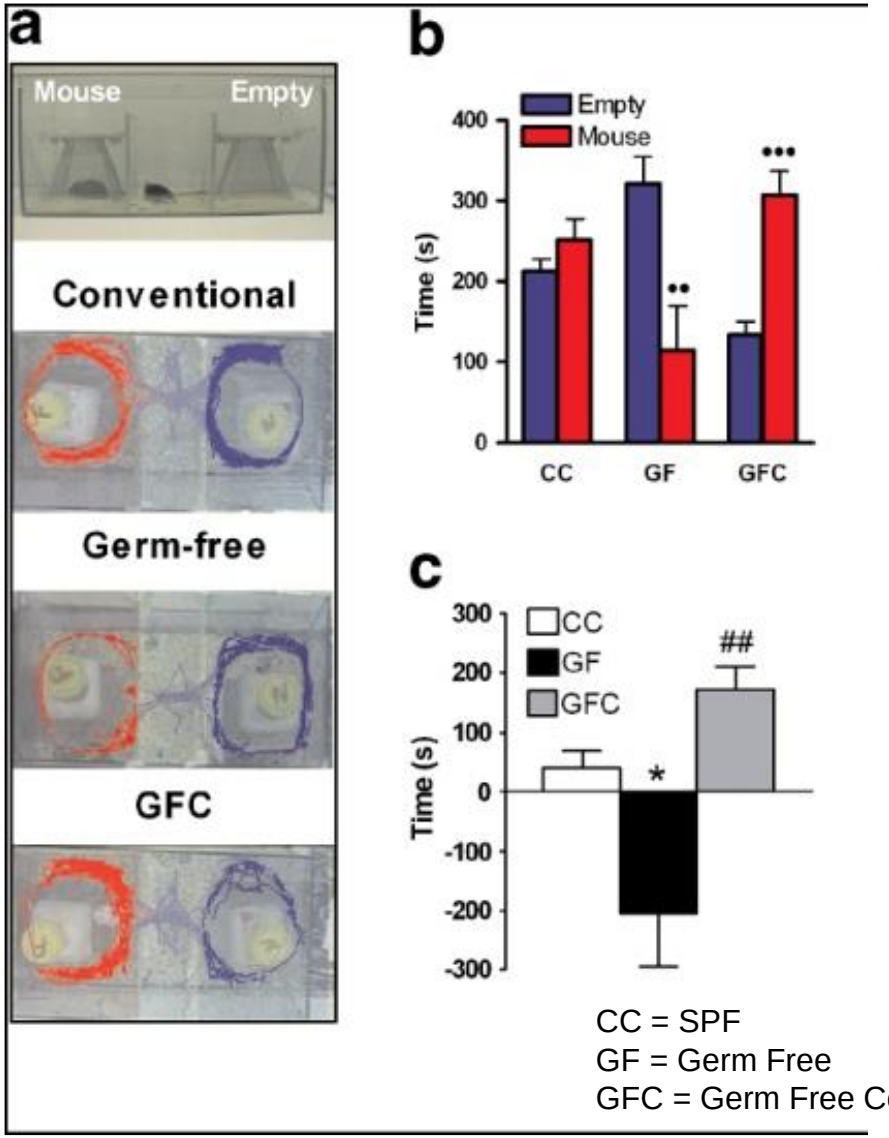
GF mice display altered social behaviour



Stimulus mouse Center Novel object



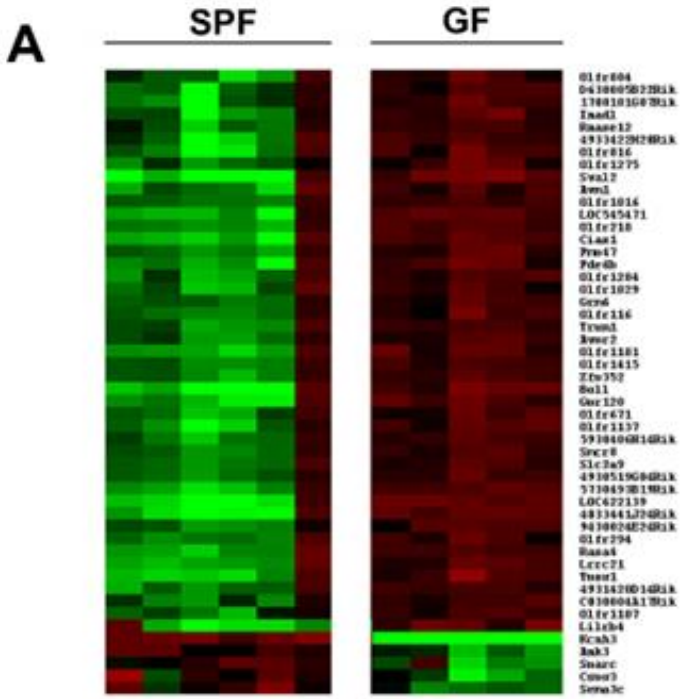
Arentsen et al 2016

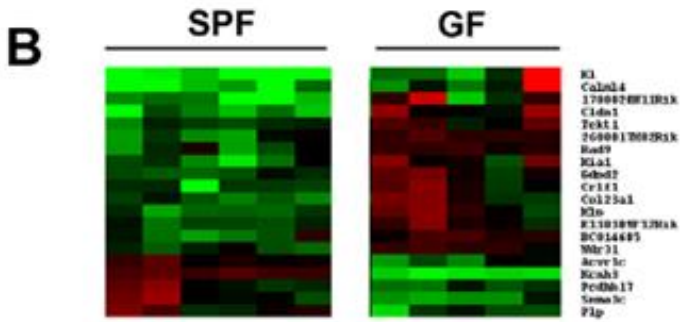


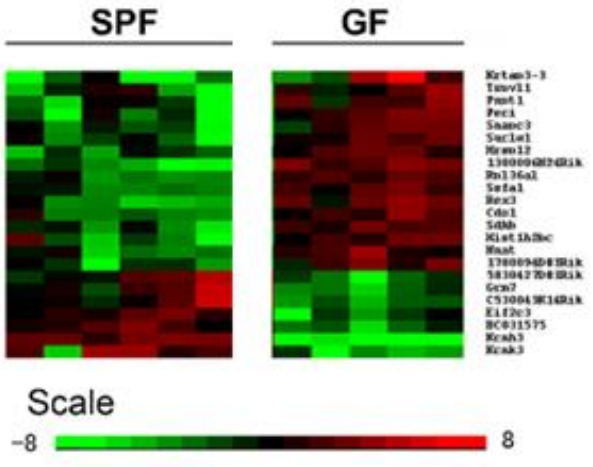
CC = SPF
GF = Germ Free
GFC = Germ Free Colonized

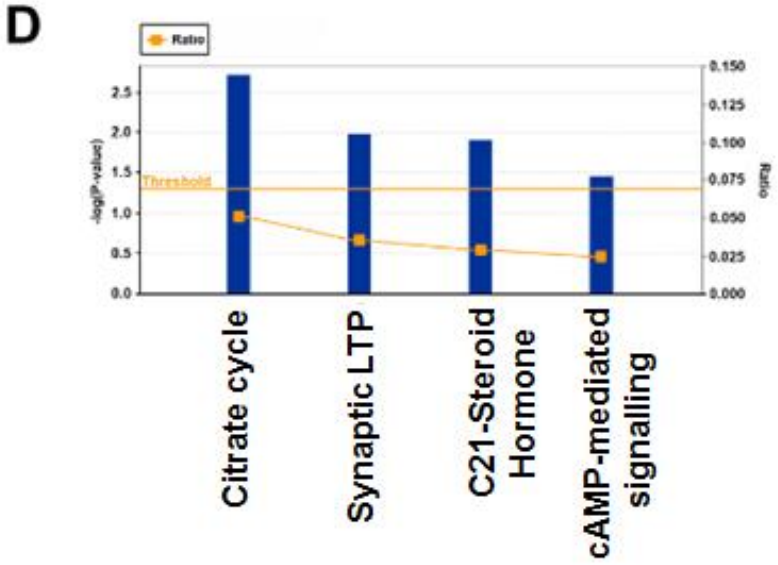
Desbonnet et al 2013

Expression profiling of GF mice and SPF mice brains



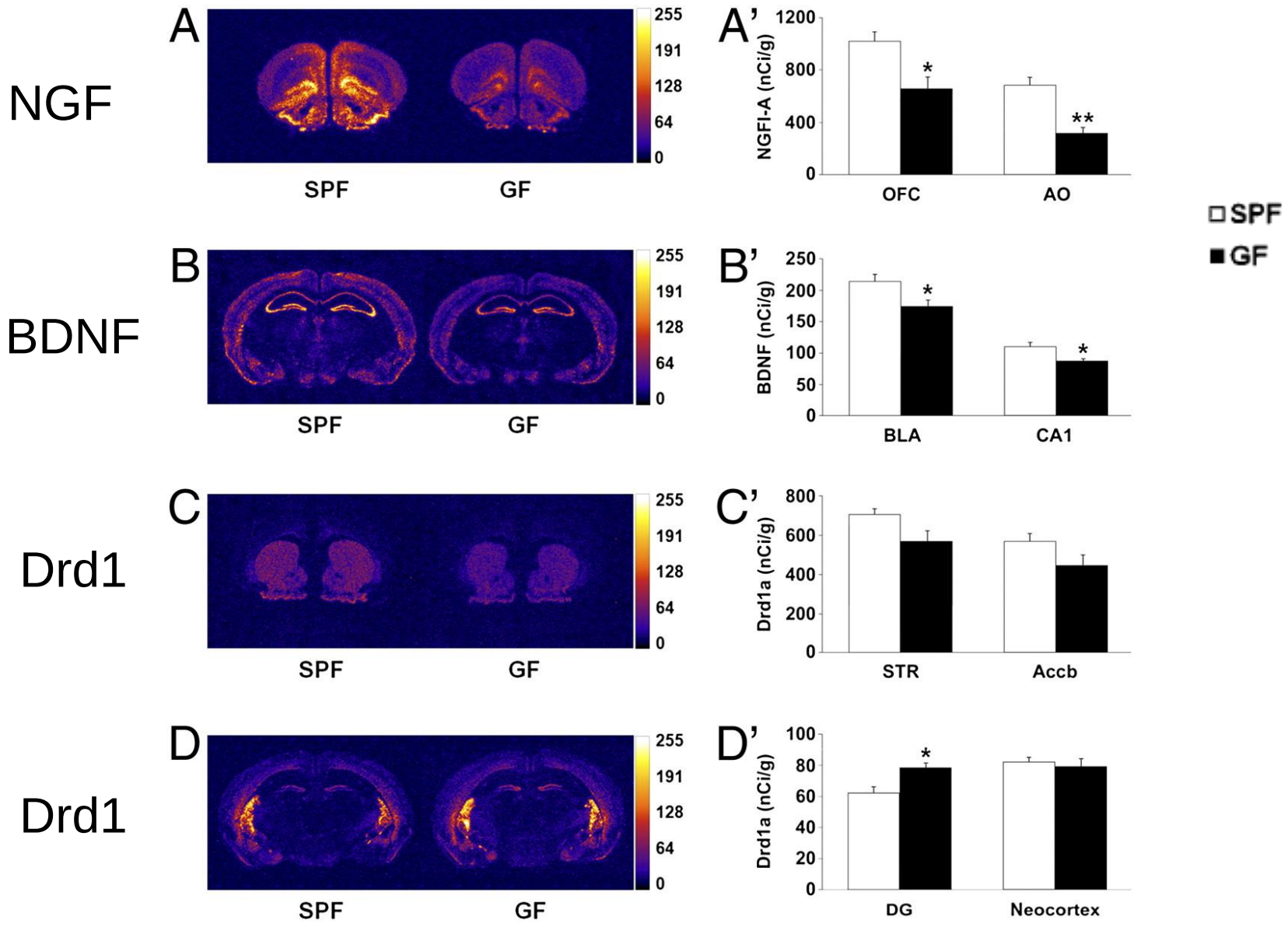






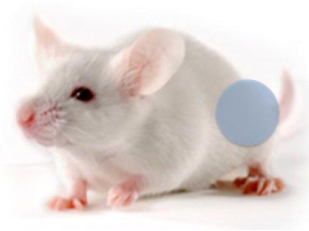
Heijtz et al. PNAS (2011)

GF mice show altered expression of synaptic plasticity related genes



Heijtz et al. PNAS (2011)

Current Findings from Germ Free Mice



Behaviour

- ↑ Motor activity
- ↑ Anxiety-like behaviour
- ↕ Social interaction

?

Brain structure & function

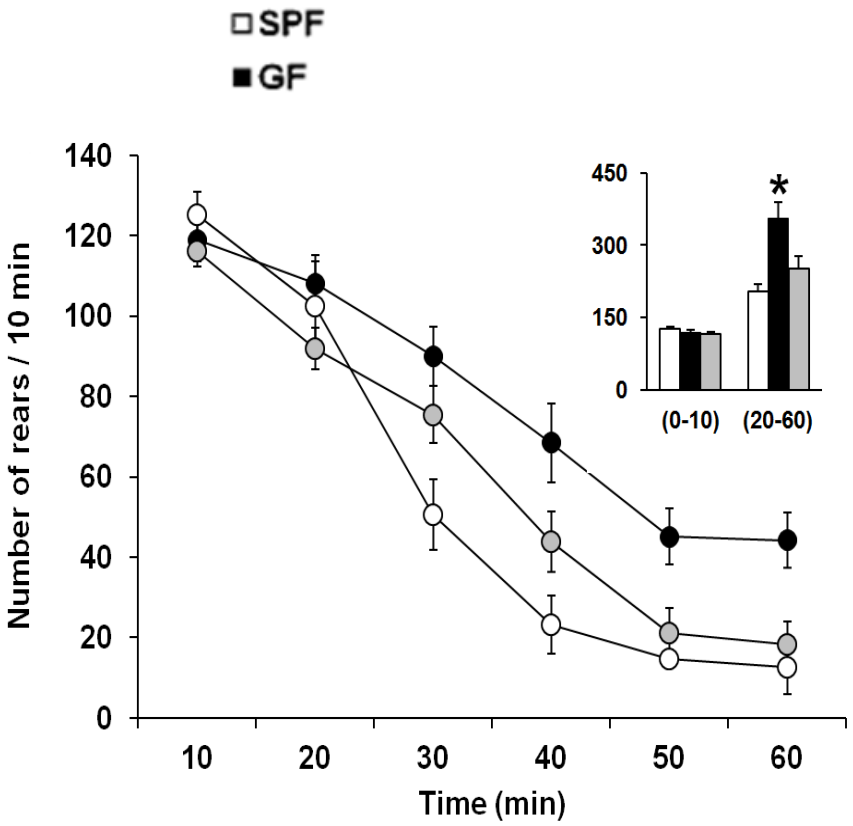
- Myelin formation
- Blood Brain Barrier
- Neurogenesis
- Spine density
- Microglia

?

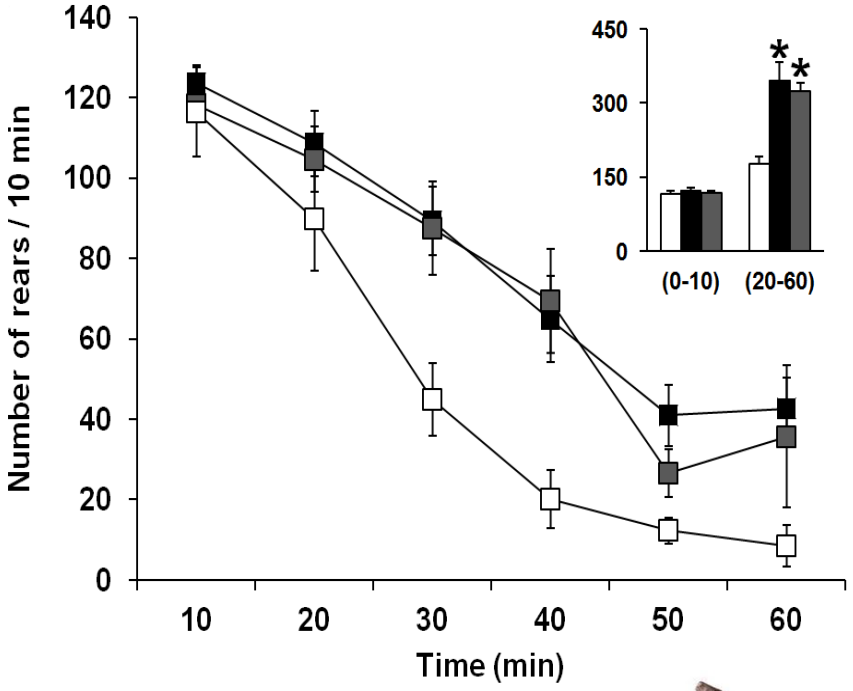
Genes

- **Synaptic-plasticity related genes**
  - PSD 95
  - Synaptophysin
  - NGF, BDNF
  - Dopamine genes
- **Kreb's cycle**
- **Steroid hormone**
- **ASD risk genes**
  - (c-MET)

Early CONV mice



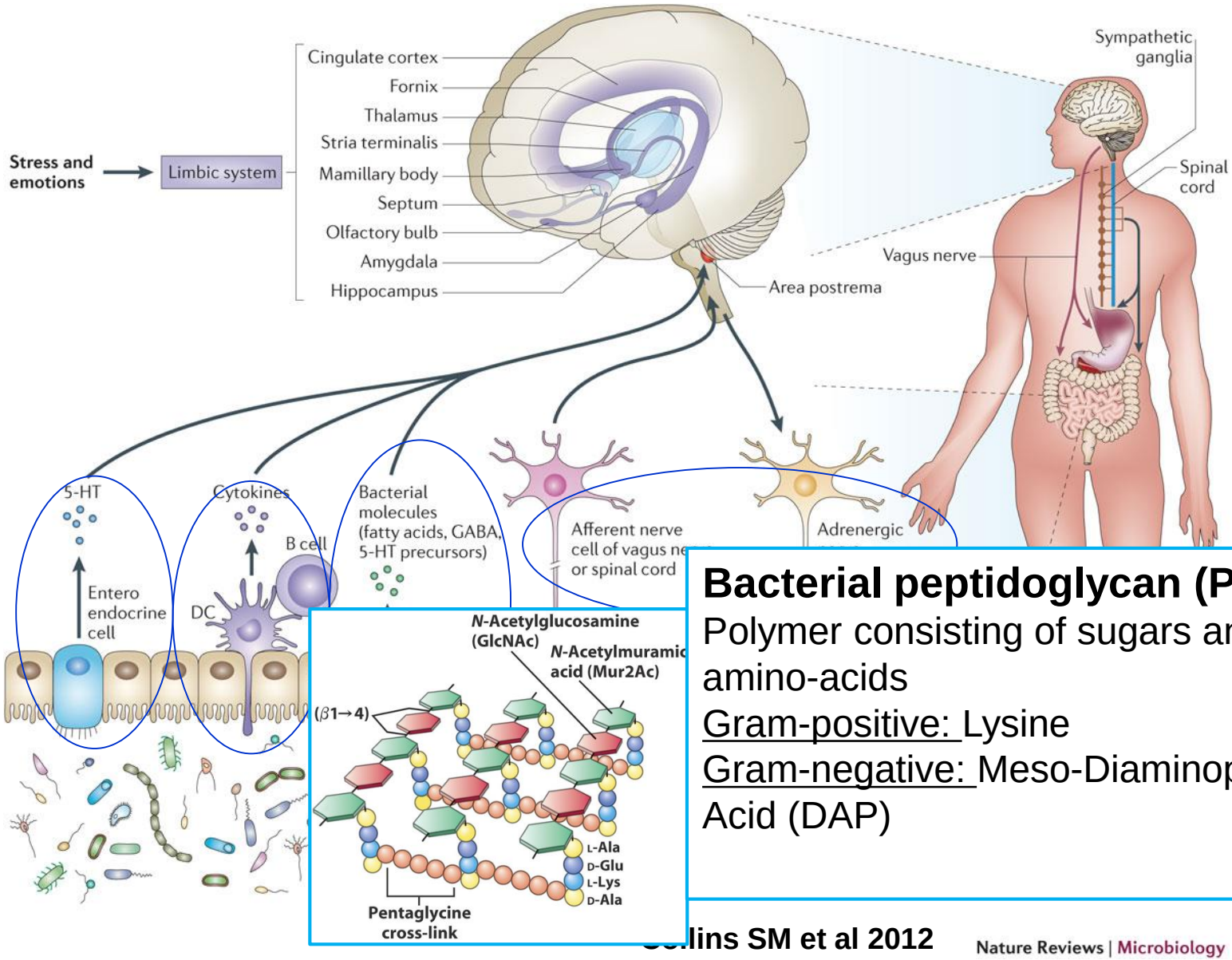
Adult CONV mice



Number of rears= number of times a mouse stands on its hind legs

Heijtz et al. PNAS (2011)

Microbiome-Gut-Brain interactions: Potential Mechanisms



Bacterial peptidoglycan (PGN)

Polymer consisting of sugars and amino-acids

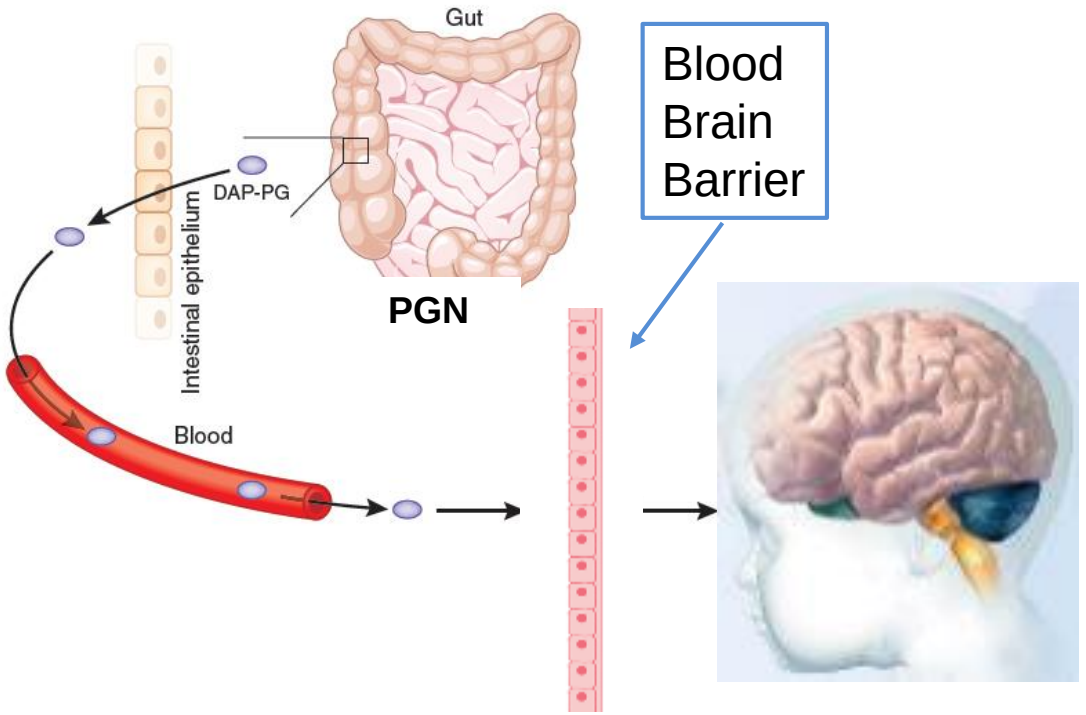
Gram-positive: Lysine

Gram-negative: Meso-Diaminopimelic Acid (DAP)

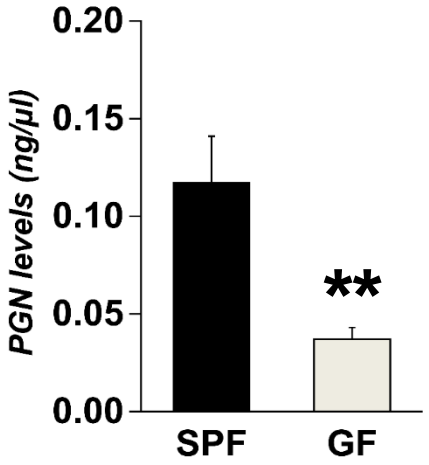
Collins SM et al 2012

Nature Reviews | Microbiology

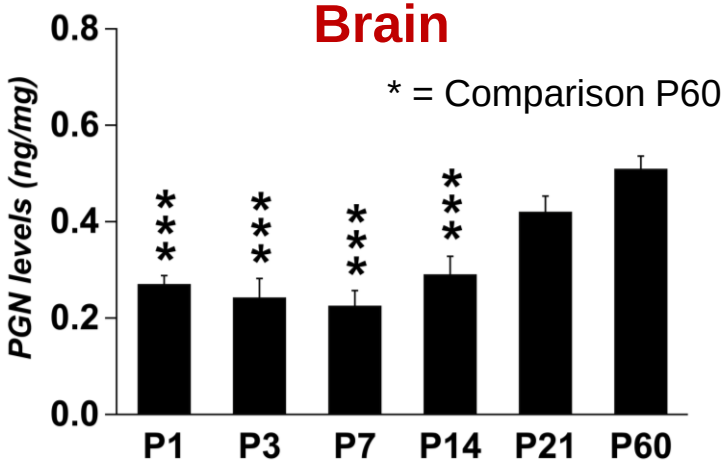
Can bacterial PGN fragments translocate from the intestinal gut mucosa into brain?



Serum



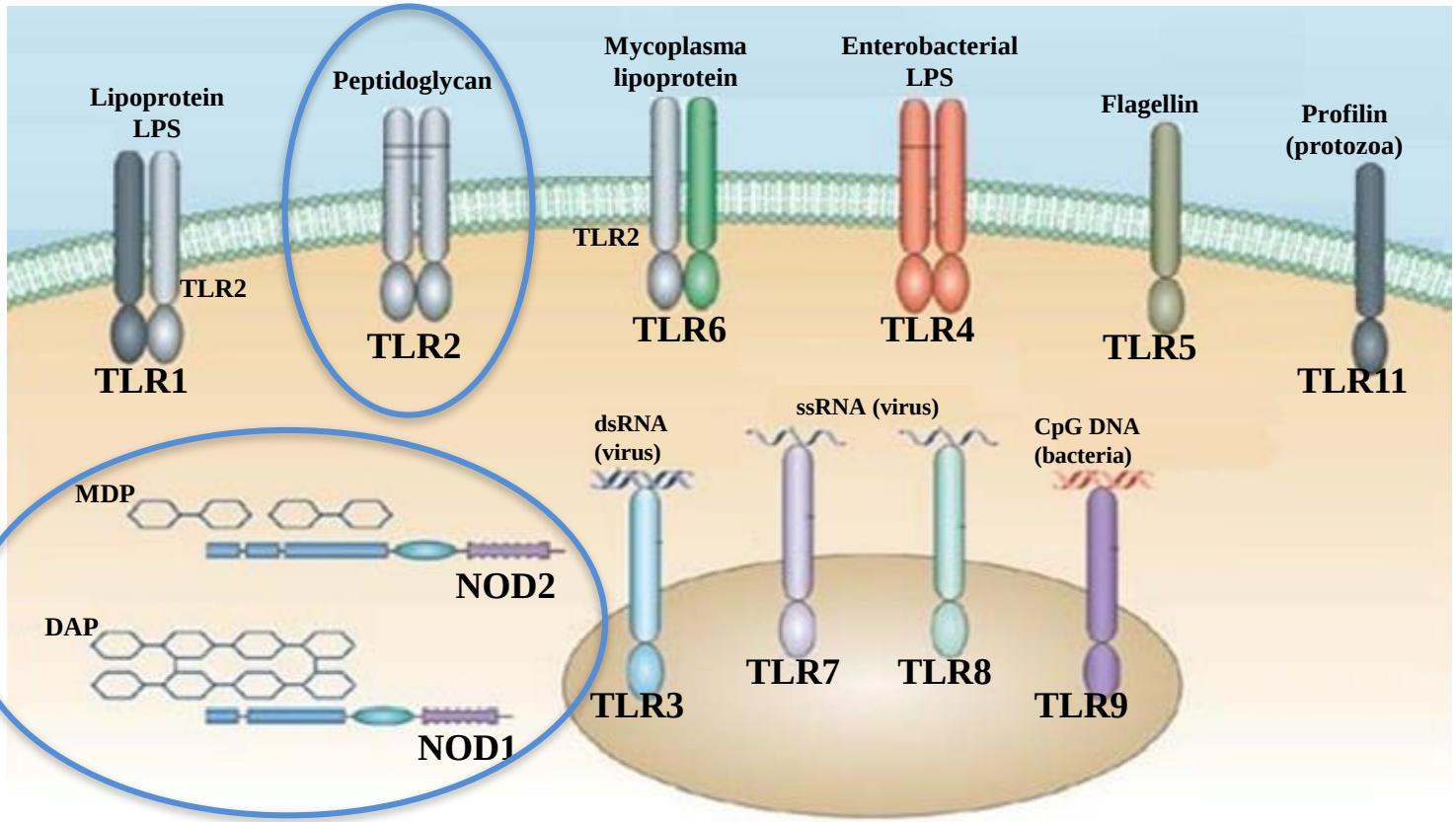
Brain

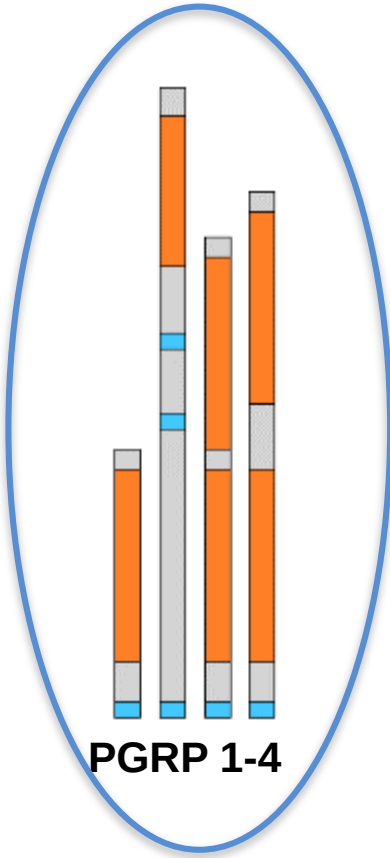


Arentsen et al, Molecular Psychiatry 2017

Pattern recognition receptors (PRR) of the innate immune system that recognize peptidoglycan (PGN)

Toll-like receptor family
NOD-like receptor family
Peptidoglycan recognition proteins

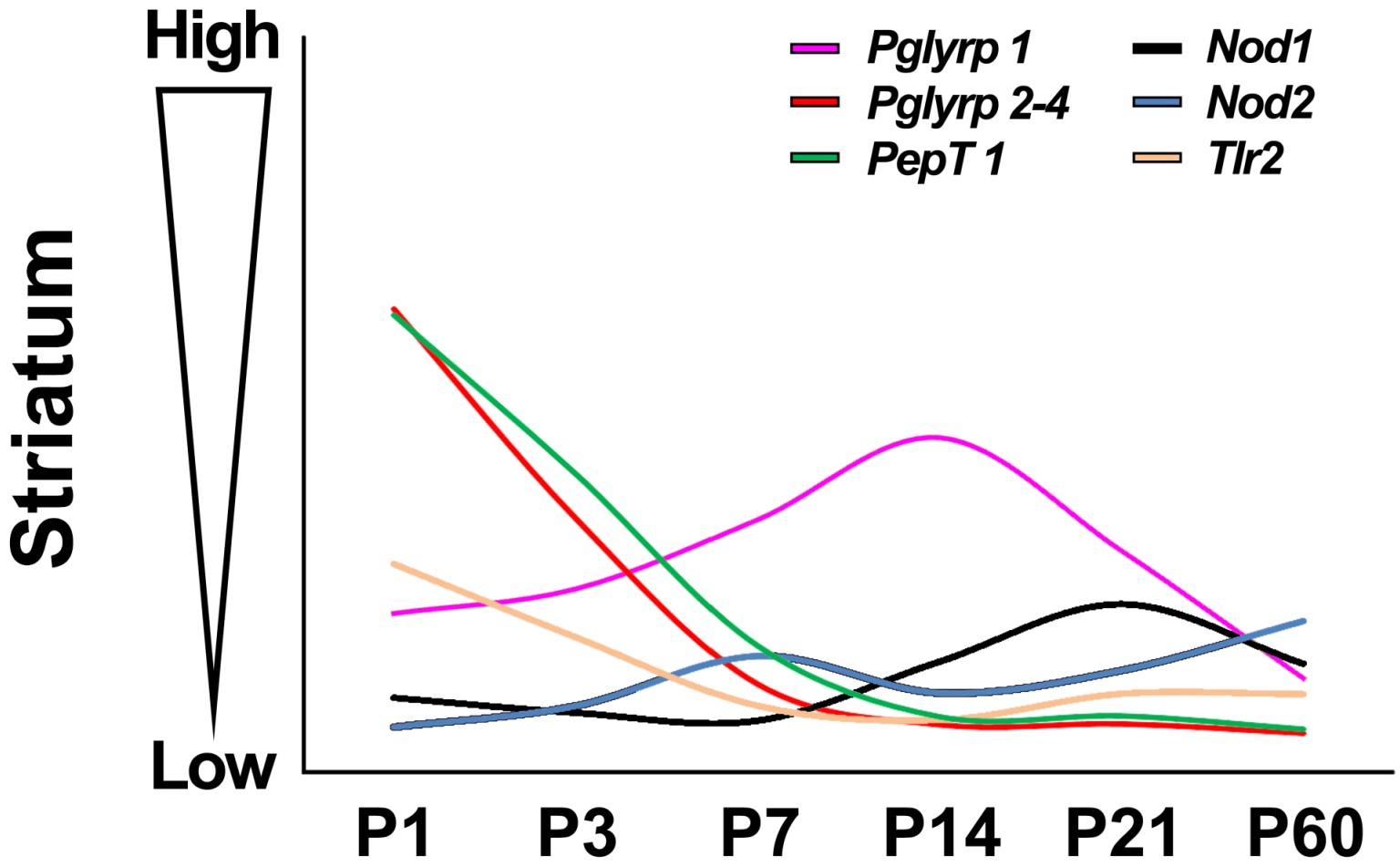




NOD: Nucleotide-binding oligomerization domain-containing protein

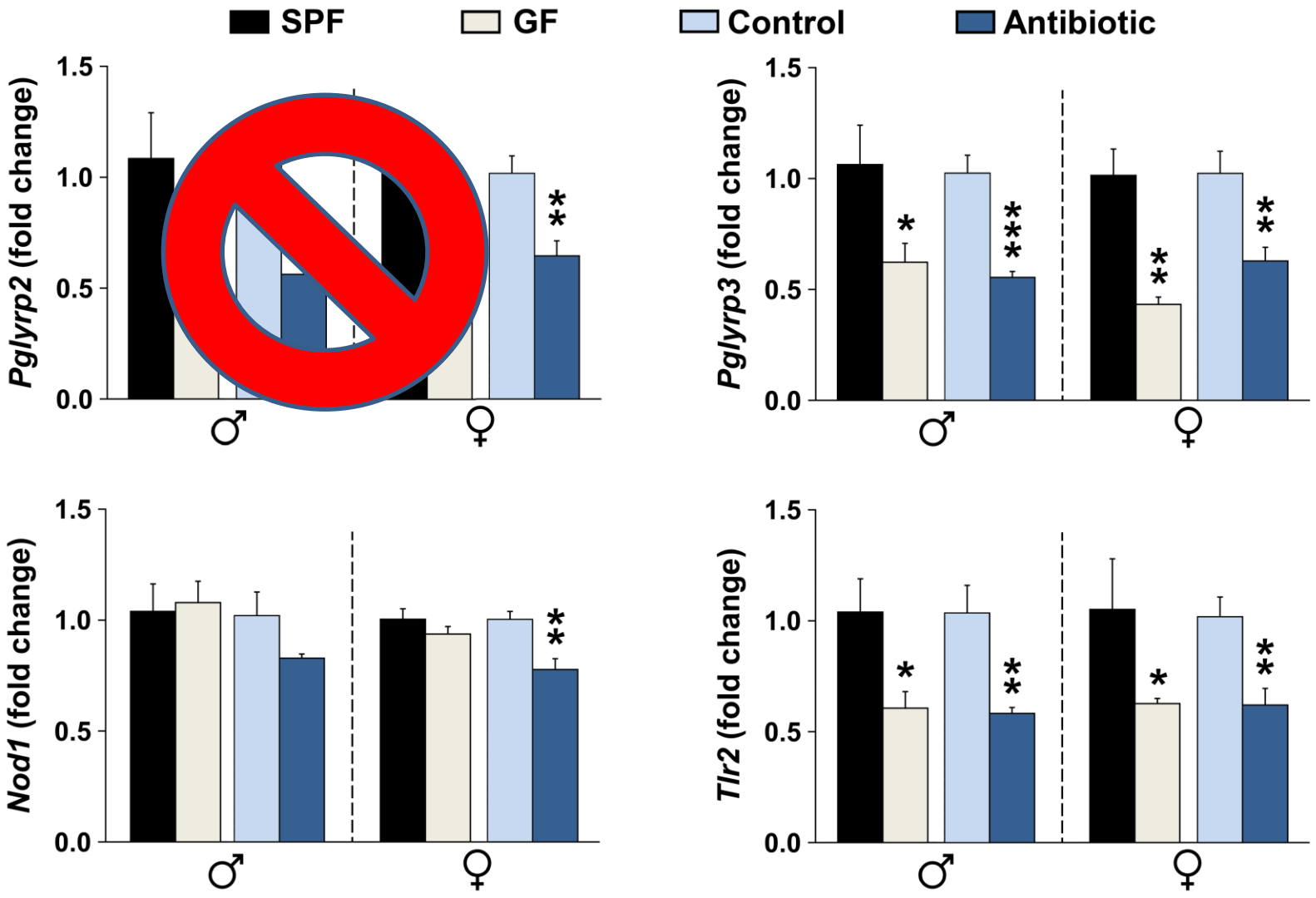
Nature Reviews | Microbiology

Are PRRs expressed in the brain during development?



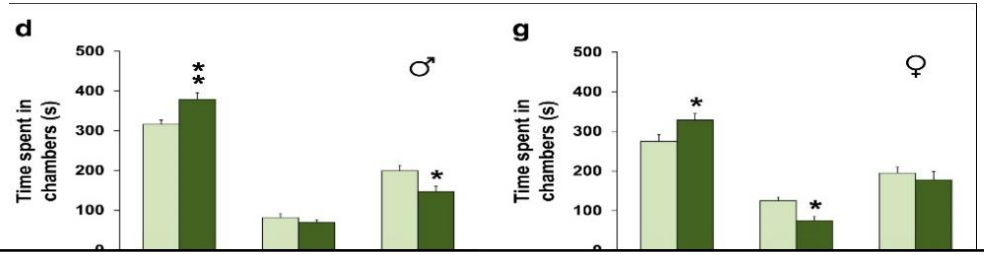
Arentsen et al, Molecular Psychiatry 2017

Does manipulation of the gut microbiota influence the expression of PRRs within the brain?



Arentsen et al, Molecular Psychiatry 2017

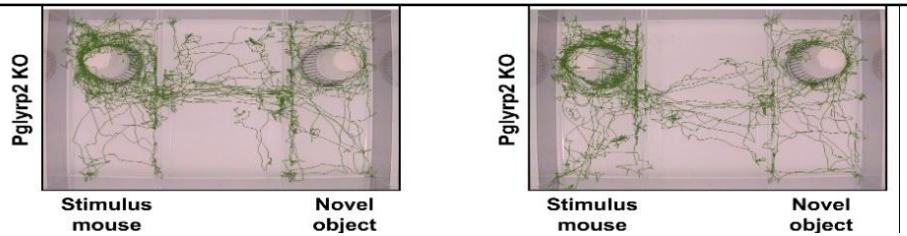
Does genetic disruption of PG2 affect social behaviour?



Absence Peptidoglycan recognition protein 2 leads to:

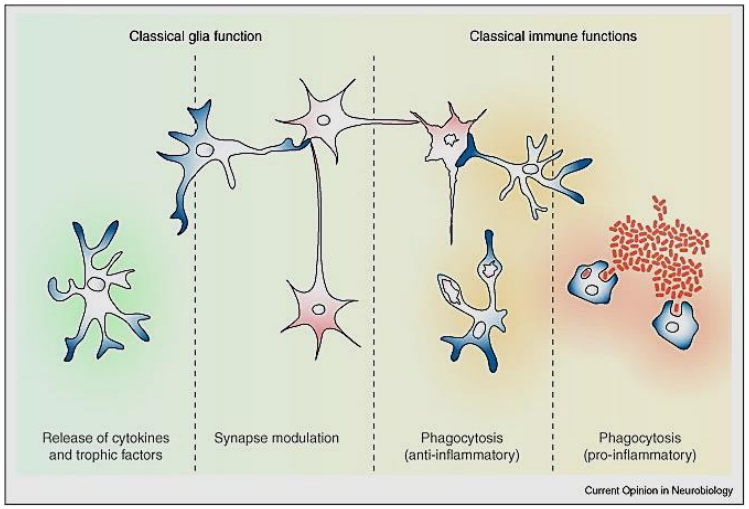
1) alterations expression of autism risk gene c-Met
2) sex-dependent changes in social behaviour

The central activation of PRRs by microbial products could be one of the signalling pathways between the gut microbiota and the developing brain.

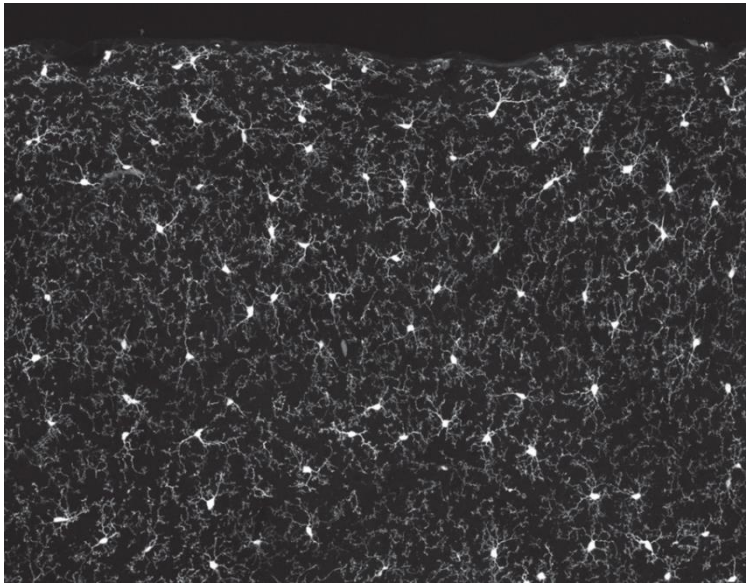


Arentsen et al, Molecular Psychiatry 2017

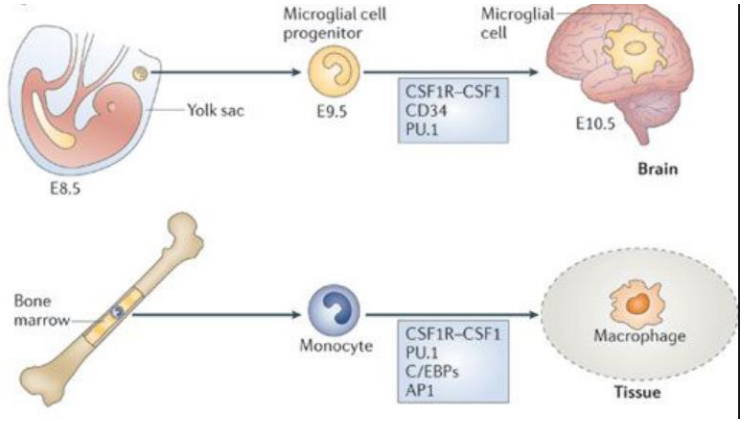
Microglia has two different functions



Microglia spread in brain



Microglia originates from yolk sac and invades brain



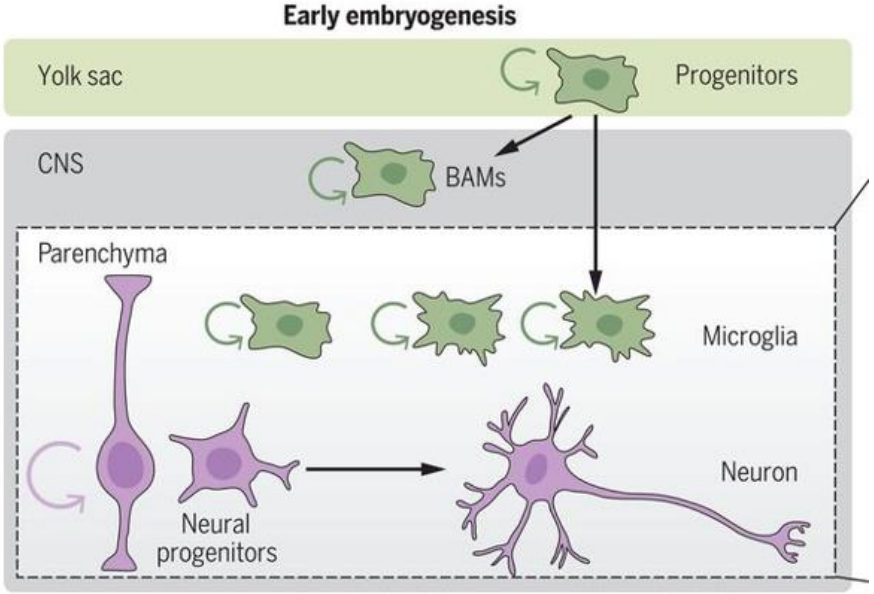
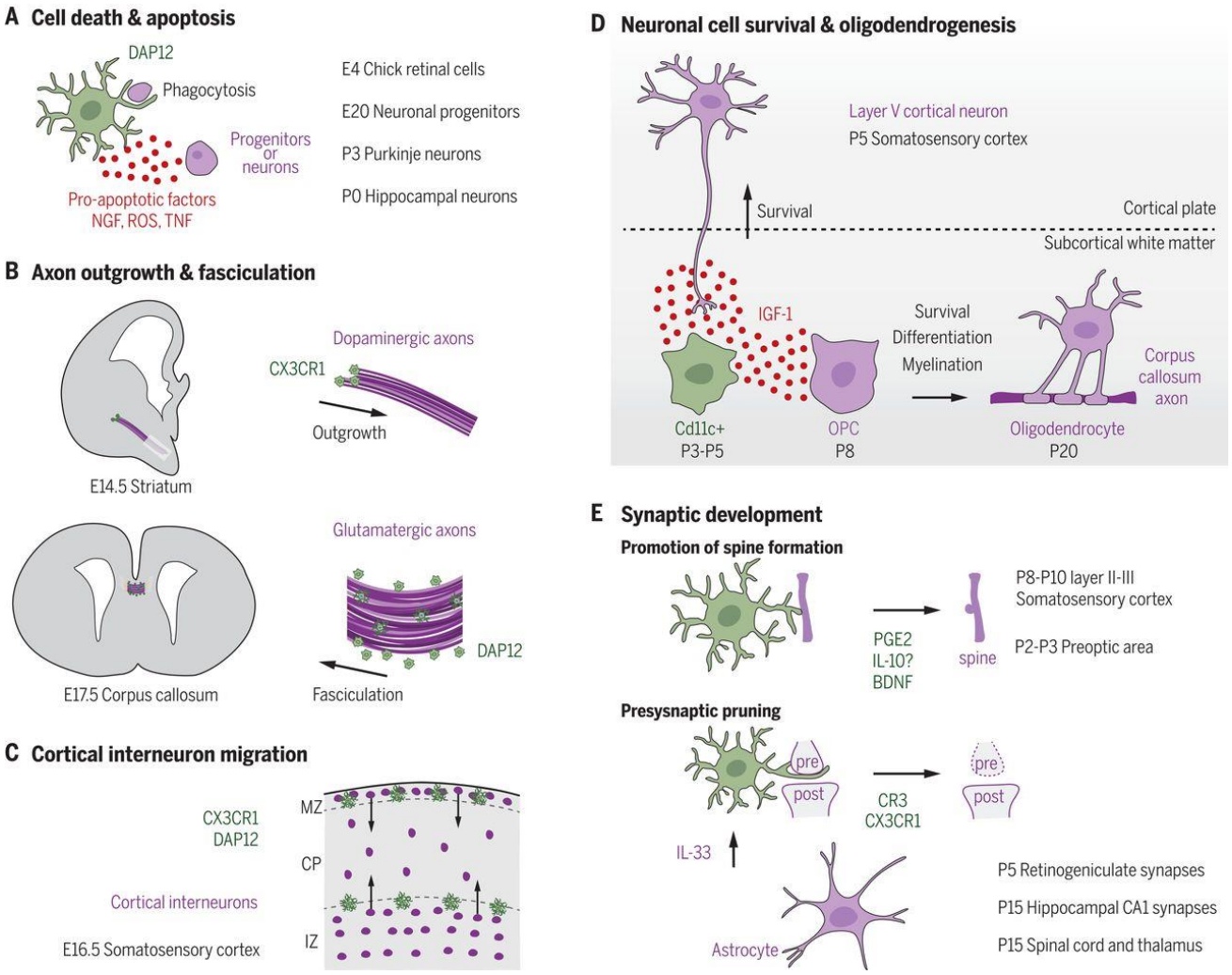
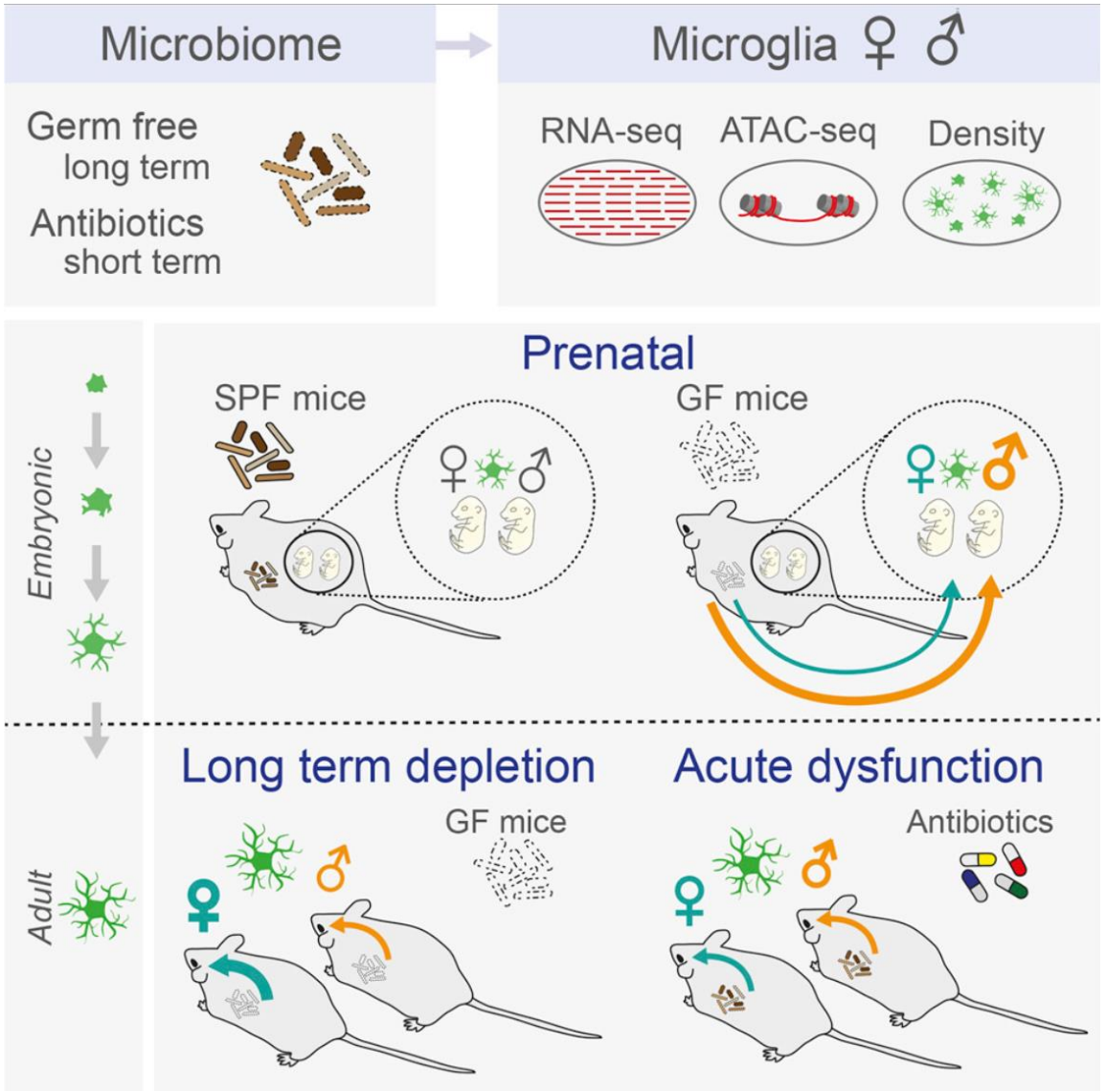


Fig. 3 Main cellular functions of embryonic and postnatal microglia.



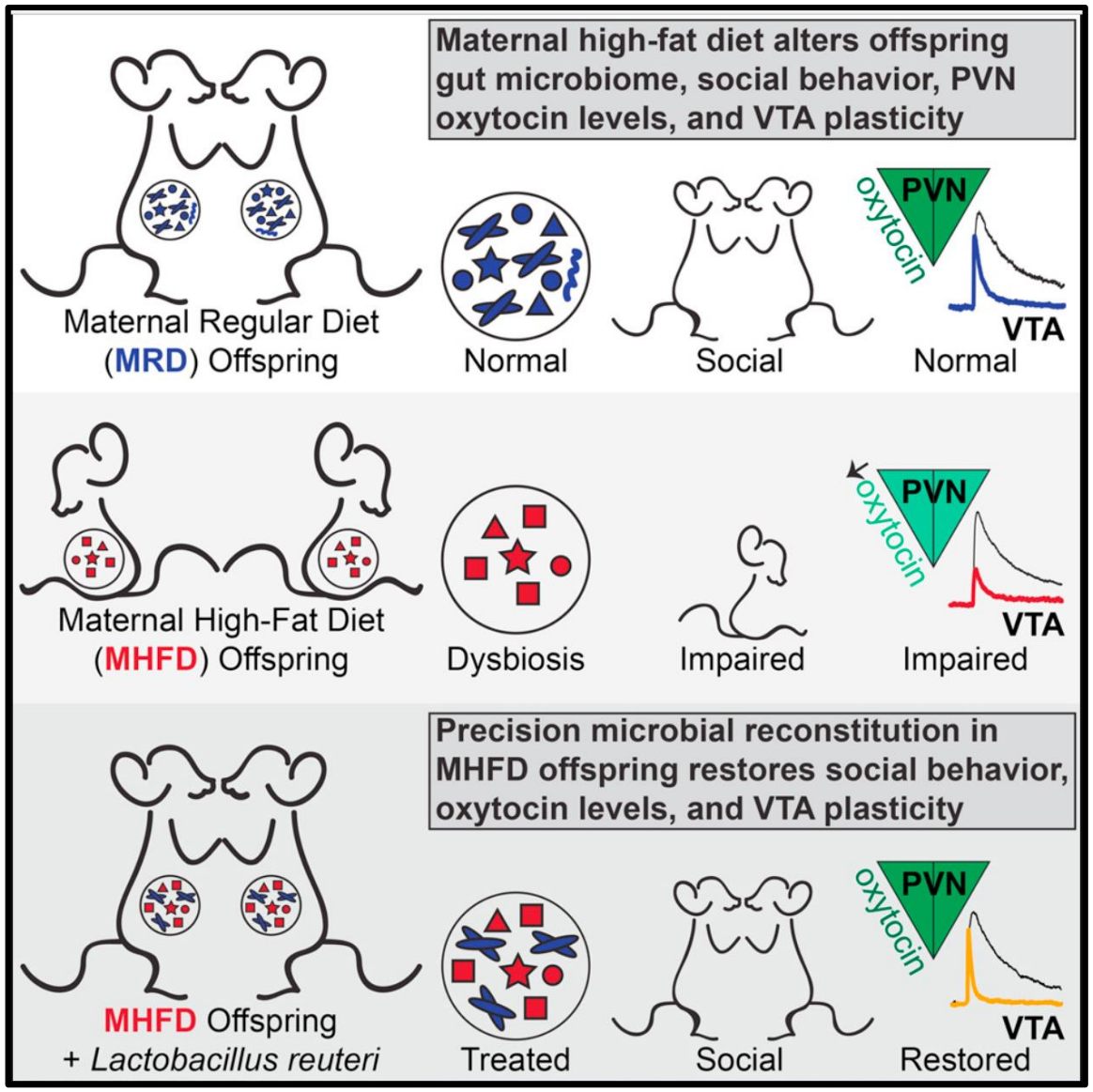
Morgane S. Thion et al. Science 2018;362:185-189

Microbiome influences prenatal and adult microglia in a sex-specific manner



Thion MS … Garel S, Cell 2018

Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring



Buffington, Di Prisco, Auchtung, Ajami, Petrosino, Costa-Mattioli 2016

Autism spectrum disorders and gastro-intestinal problems

- **Prevalent GI-problems**
  - → Diarrhea/constipation, gastric reflux, abdominal pain
  - → Affected gut epithelium and intestinal permeability
- **Correlation of GI symptoms with autism severity**
- **Excessive use of oral antibiotics**
- **Potential effect of probiotics**
- **Atypical gut microbiota**
  - → > *Clostridium, Lactobacillus, Desulfovibrio*
  - → < *Bacteroidetes/Firmicutes ratio*
- **Immune alterations**
  - → Microglia activation, altered cytokine profile

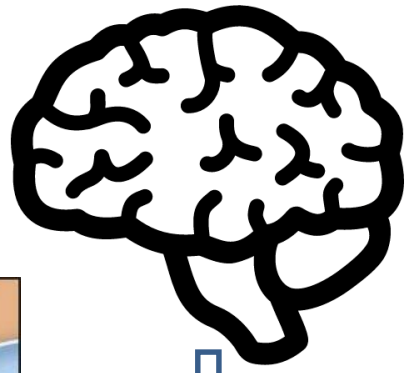
Human Brain Development

Genes

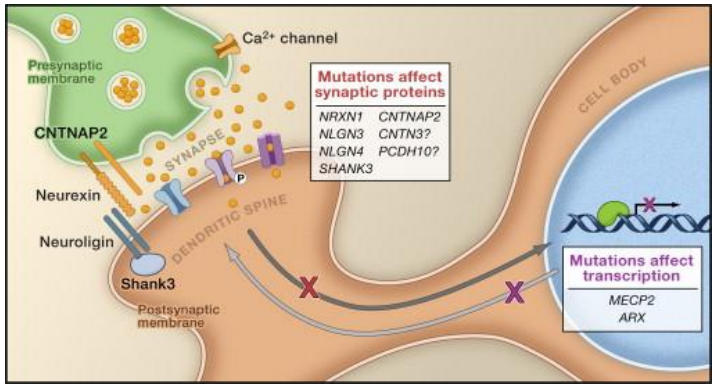
Epigenetics

Environment

Brain



Heritability 50-83%



Autism SD

Maternal infections

Early viral
Late bacterial
Elev. cytokines amniotic fl.

Danish medical birth register

⇨ early activation of the immune system

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Anaerobes in the microbiome

Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders

Table 4
Species level phylotypes significantly different after multiple testing correction.

Taxonomic assignment (family/genus/species)[a]	Two-tailed Mann-Whitney *U* test		Median[b] (25%/75%)	
	p value	adjusted *p*	Neurotypical (n=21)	ASD (n=23)
Pasteurellaceae/Haemophilus/parainfluenzae	<0.001	0.006	0.01 (<0.01/0.06)	0 (0/0)
Ruminococcaceae/Faecalibacterium/prausnitzii	<0.001	0.02	8.84 (5.62/12.97)	1.06 (0.53/3.41)

[a] Detailed information with a whole list of species level phylotypes are listed in the Dataset S1.
[b] Unit: the percentile (%) abundance from a total bacteria.

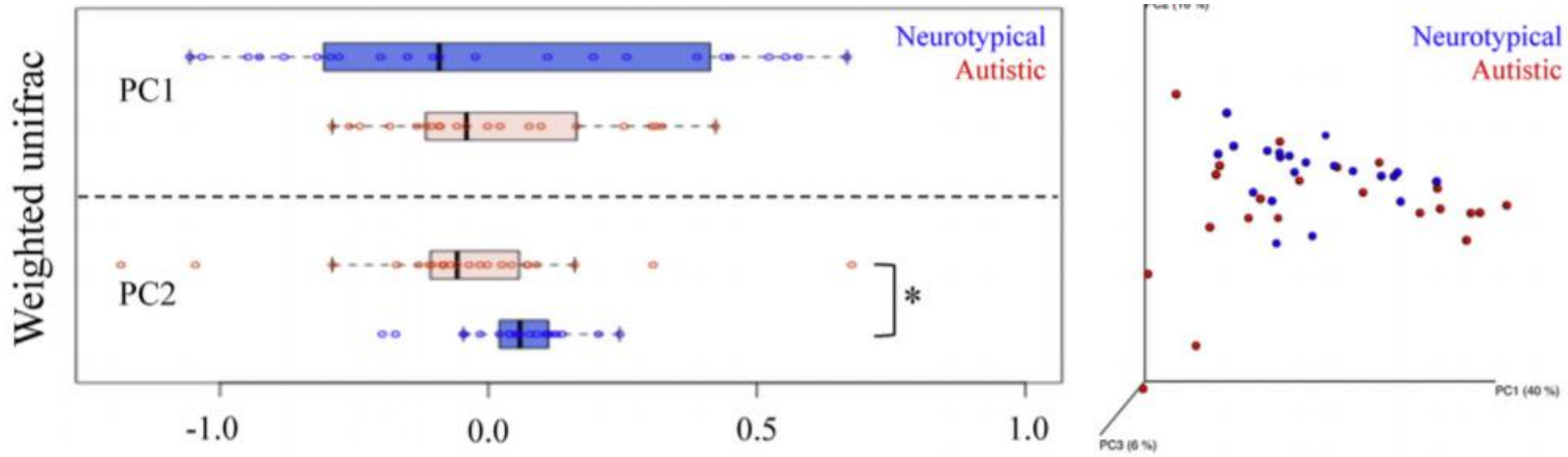


Fig. 4. Principal coordinate axis values (PC1 and PC2) and corresponding 3-dimensional PCoA plots based on unweighted (above) and weighted (below) UniFrac analyses. * and ** indicates two-tailed Mann-Whitney *U* test *p* values less than 0.05 and 0.0005, respectively.

Kang *et al. Microbiome* (2017) 5:10
DOI 10.1186/s40168-016-0225-7

Microbiome

RESEARCH Open Access

Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study

- **80% reduction of GI symptoms**
- **Improvements of behavioural ASD symptoms**
- **Engraftment of donor microbiota and beneficial changes of gut environment**

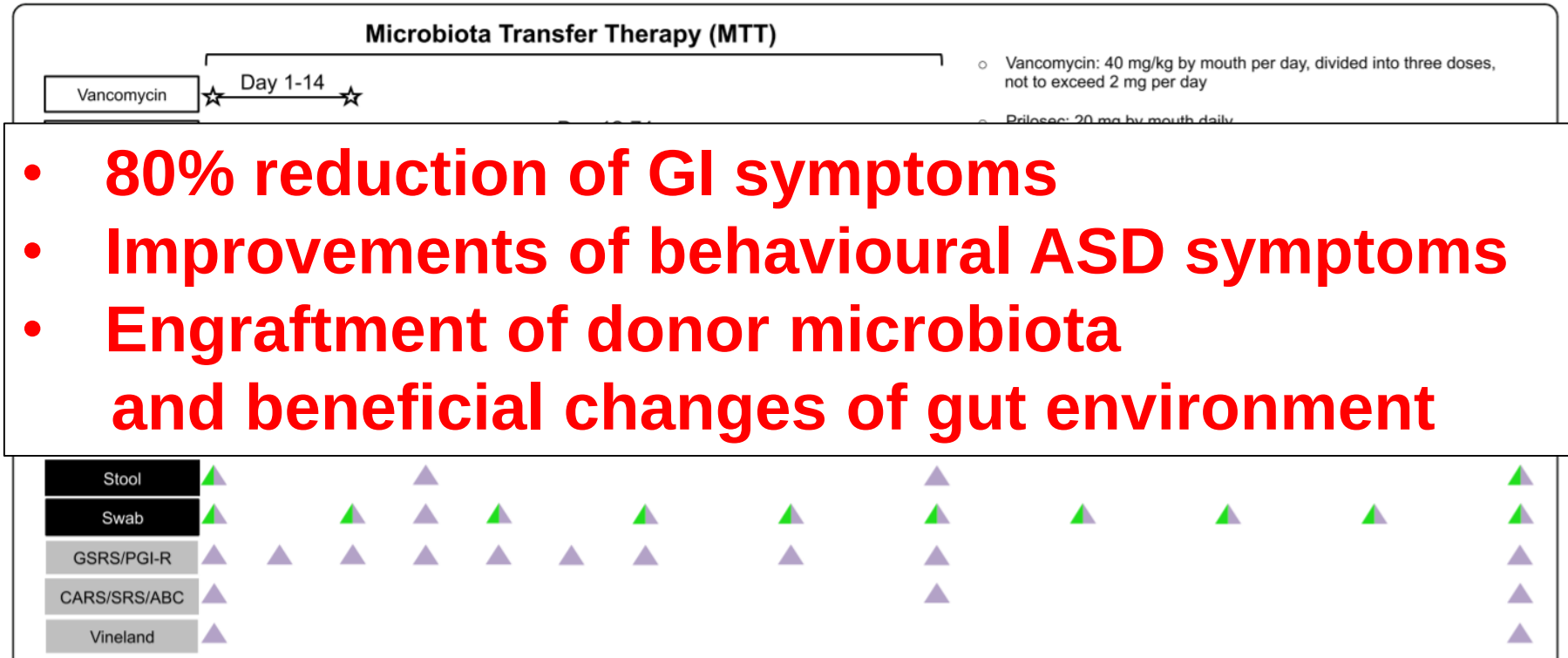
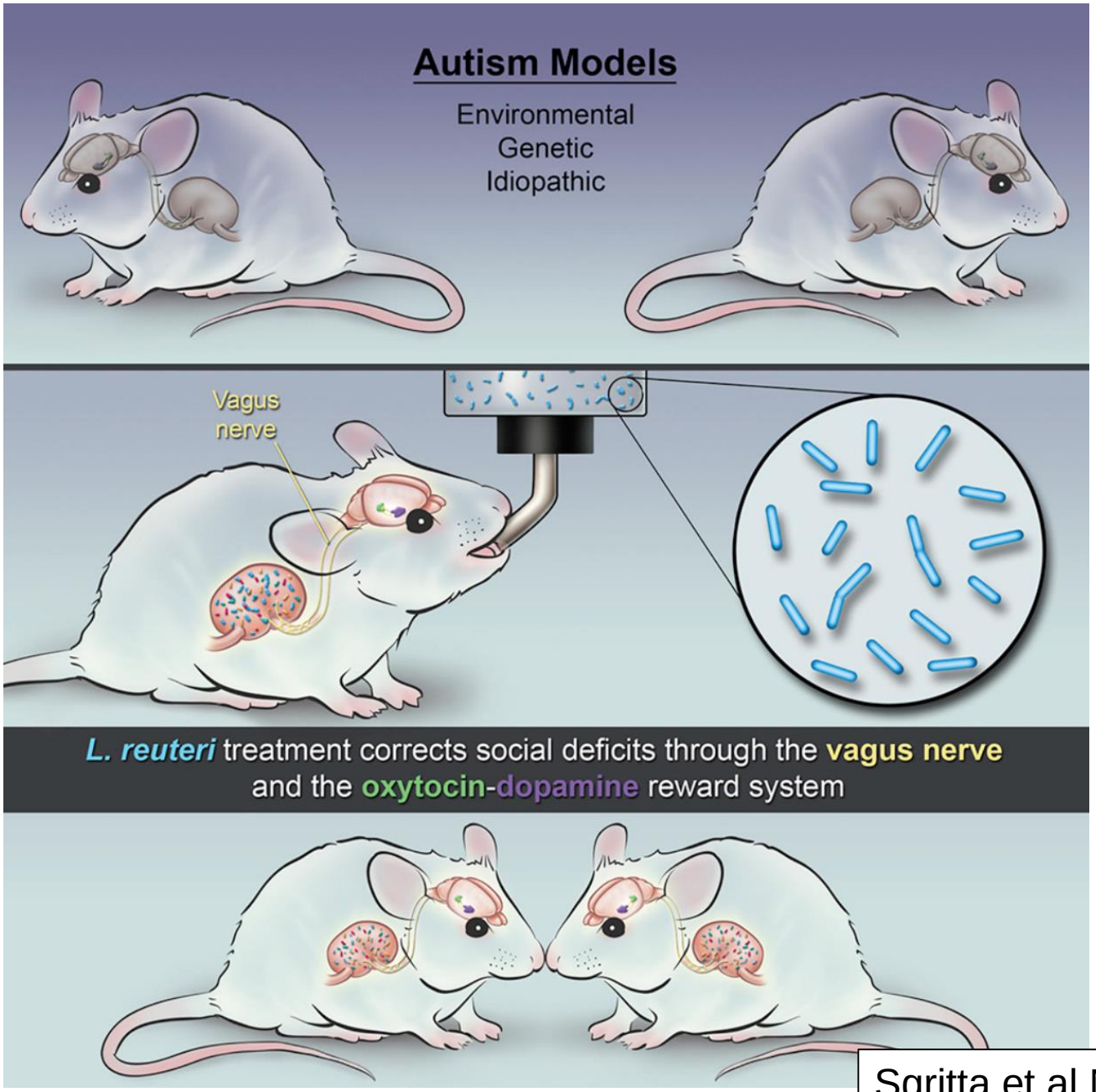


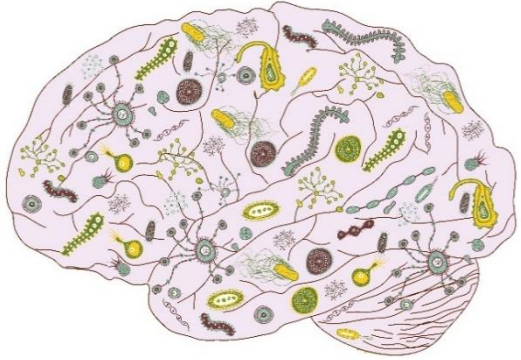
Fig. 1 Study design timeline. The trial consists of 10-week Microbiota Transfer Therapy (MTT) and 8-week follow-up observation period after treatment stopped. Schematic timeline represents a series of treatments that were performed during MTT (*top*) and frequencies of sample collection and GI/behavior assessments (*bottom*; neurotypical and ASD group colored in *green* and *purple*, respectively)

Lactobacillus reuteri can rescue social behaviour in ASD mouse models



Sgritta et al Neuron 2018

Summary



1. 240 million children worldwide are at risk not reaching their developmental potential due to poverty and undernutrition
2. Gut microbiota (child and pregnant mother) influence development
3. Experimental studies (gnotobiotic mice) show that microbiota influences behaviour, brain structure & function, and gene expression
4. Early programming - critical period gives life long outcome
5. Several gut-brain pathways
6. Antibiotics reduces diversity and influence cognitive development mood, and behaviour
7. Emerging role for gut microbiome in Autism Spectrum Disorder and other neurodevelopmental disorders

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  - →**Rochellys Diaz Heijtz**
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- Canada
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