Microbiota -Gut-Brain interaction and synaptic maturation

Silvia Di Angelantonio


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MICROBIOTA

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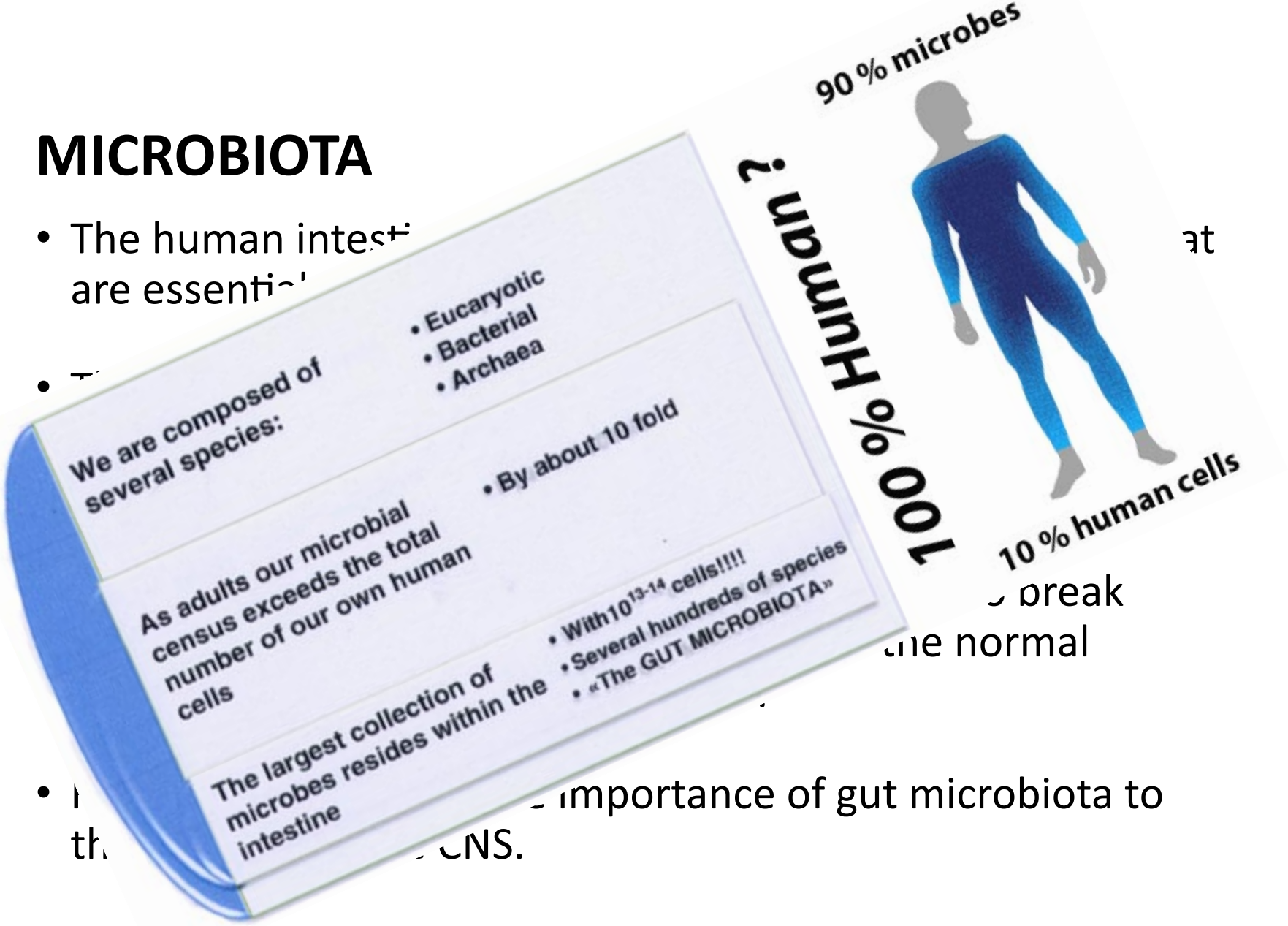


MICROBIOTA:

The human intestine harbors nearly 100 trillion bacteria that are essential for health. The largest microbial component of the human microbiome is located in the large intestine of the gastrointestinal (GI) tract.

- critical contributions to metabolism by helping to break down complex polysaccharides
- critical to the normal development of the immune system.

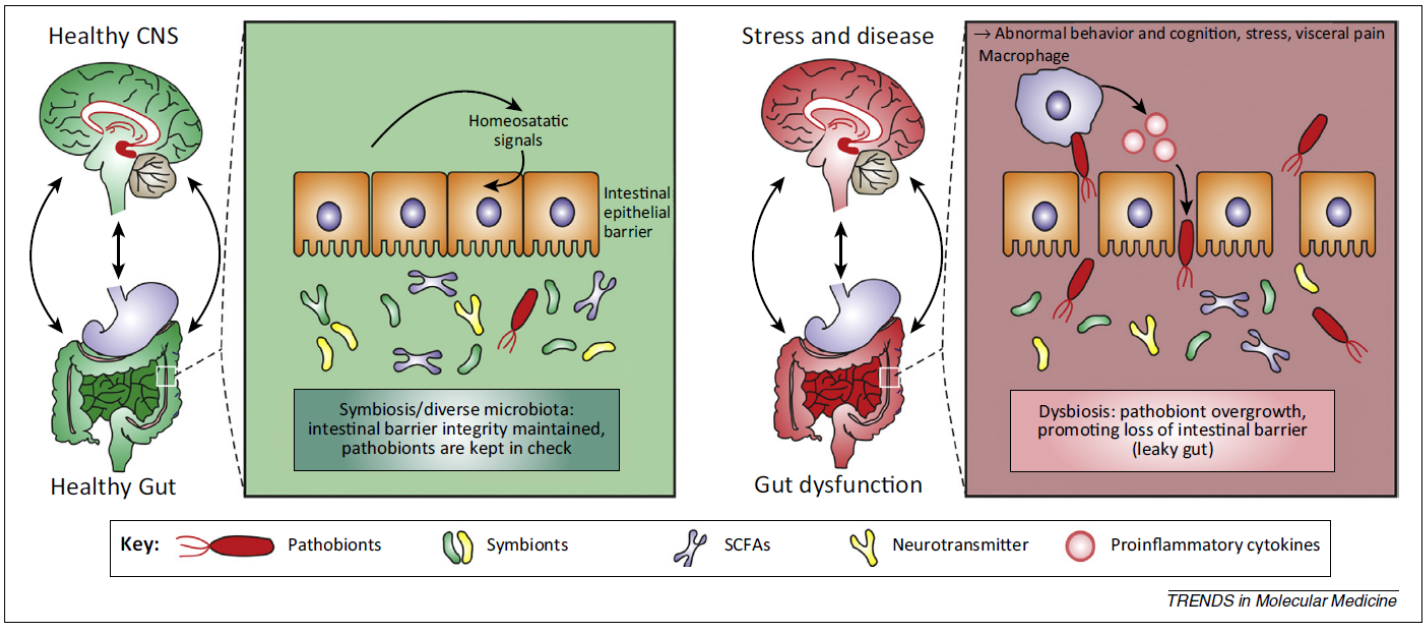
Recent studies reveal the importance of gut microbiota to the function of the CNS.

MICROBIOTA–GUT–BRAIN AXIS:

A complex network of communication between the gut, the intestinal microbiota, and the brain, modulating

- immune
-GI
-and CNS functions.

It encompasses the CNS, the sympathetic and parasympathetic branches of the autonomic nervous system, as well as the enteric nervous system and the neuroendocrine and neuroimmune systems.



In healthy individuals:
the normal dominant microbiota is relatively stable and forms a mutually beneficial rapport with the host.

Perturbations may have serious consequences and has the potential to exacerbate brain, digestive, and metabolic disorders.

Bidirectional communication between the microbiota and the CNS influences stress reactivity, pain perception, neurochemistry, and several brain– gut axis disorders.

The composition of the gut microbiota during critical periods of CNS development is affected by a broad range of factors. Perturbation of any of these factors can lead to host stress or disease.

MICROBIOTA DEVELOPMENT

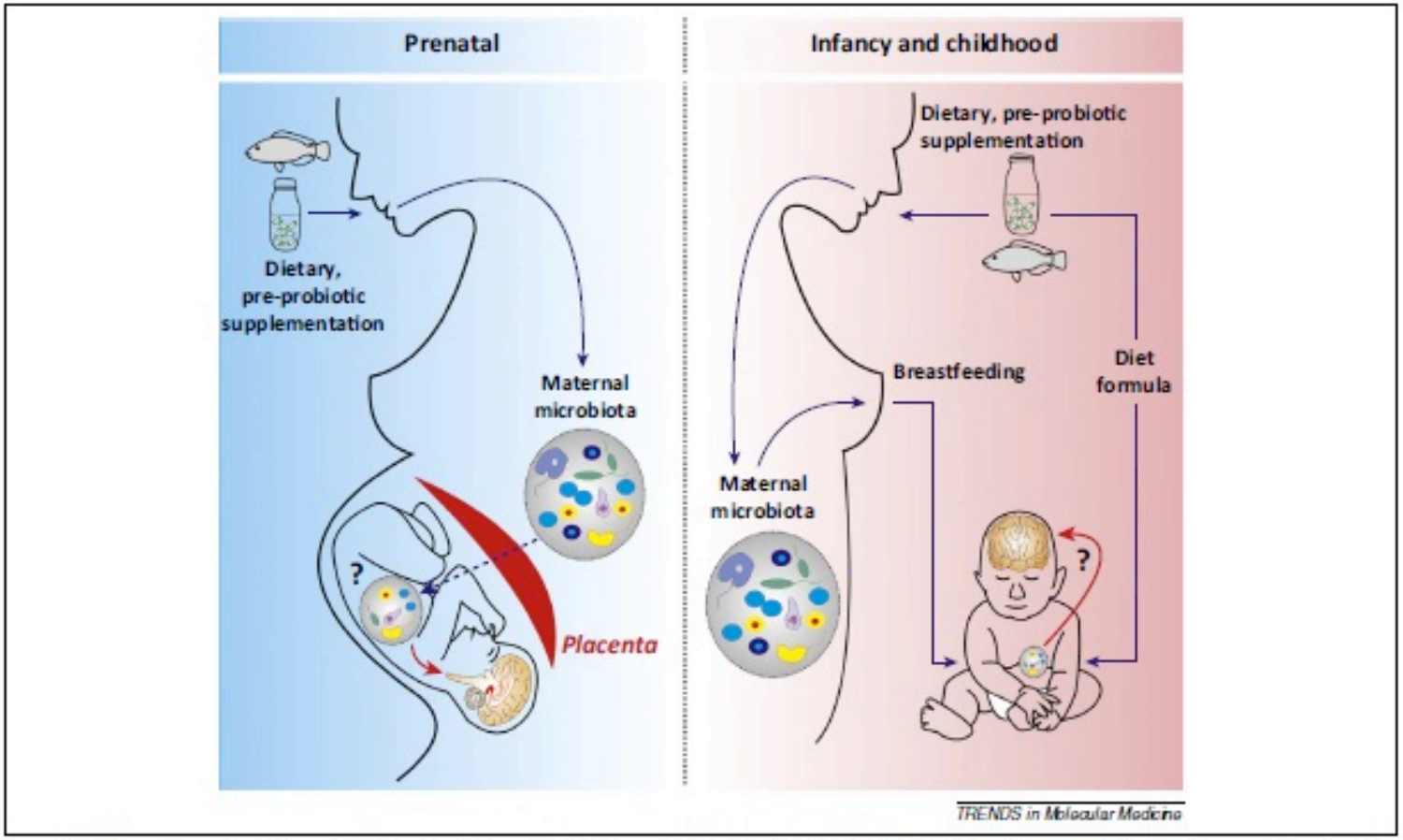


Figure 3. Windows of opportunity to modulate the microbiome of the infant prenatally and postnatally. Microbiota–gut–brain communication during prenatal and postnatal development is shown. Although still controversial, some evidence suggests that the microbiota of the infant before birth is not sterile, but may be influenced by the maternal immune state and nutrition. Prenatal and postnatal development undergoes vigorous neurodevelopmental phases and it is possible that it may be indirectly influenced by the fetal microbial population (via microbiota of the mother). This opens avenues for the development of novel dietary and microbe-modulating therapies, which may directly and indirectly alter the composition of the microbiota and neurodevelopment of the infant.

MICROBIOTA DEVELOPMENT

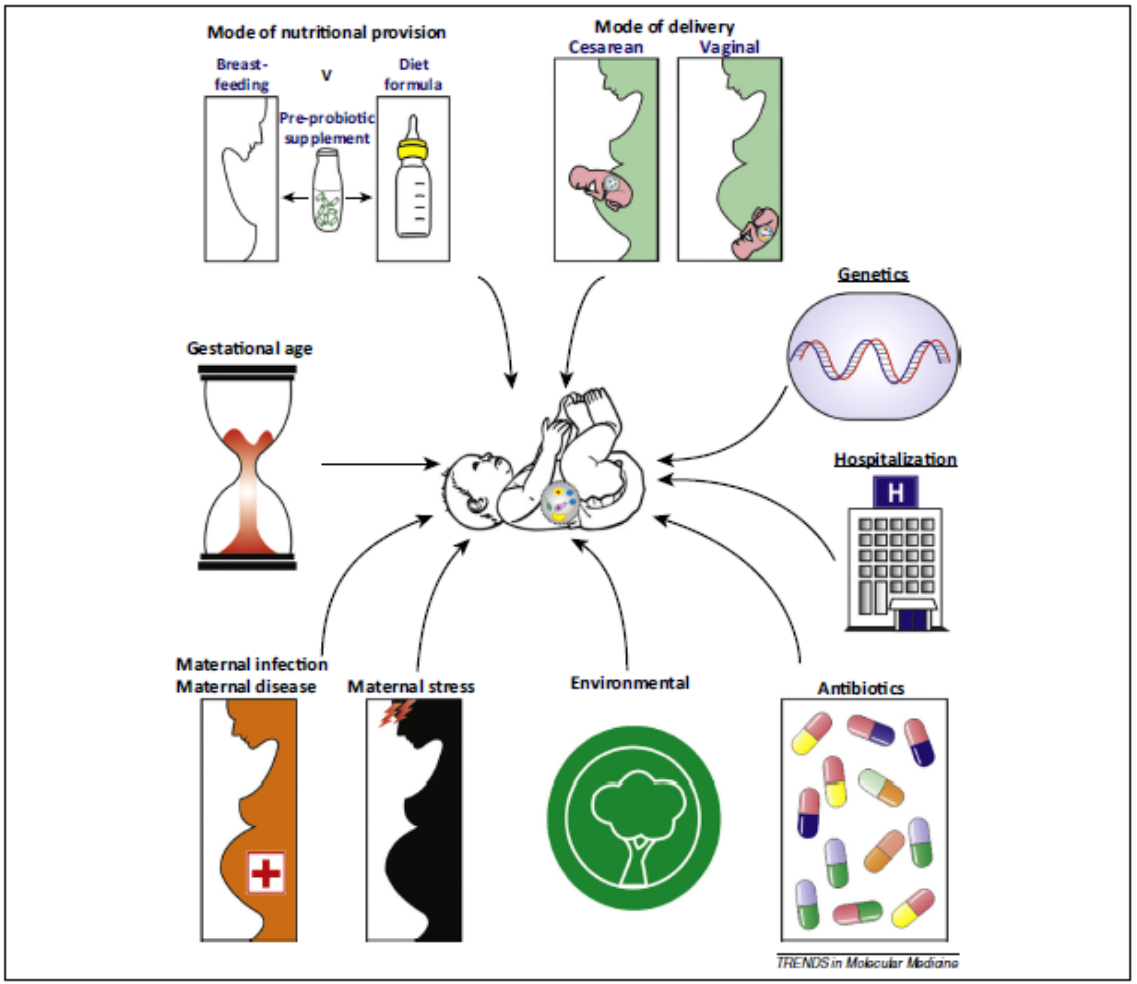


Figure 4. Factors influencing the development of the infant microbiota. Several factors play a role in shaping of the bacterial landscape in the development of the infant microbiota. In addition to mode of birth, mode of early nutrition, environment, other factors such as gestational age, genetics, and hospitalization, also influence the microbial composition of the infant. Infections (both maternal and infant) and antibiotic usage influence the trajectory of the developing microbiota as does the selective transient enrichment by probiotics and prebiotics. Taken together, such a plethora of factors with the ability to modulate the microbiota development suggest the importance of environmental influence superimposed over genetics in the establishment of a core microbiome.

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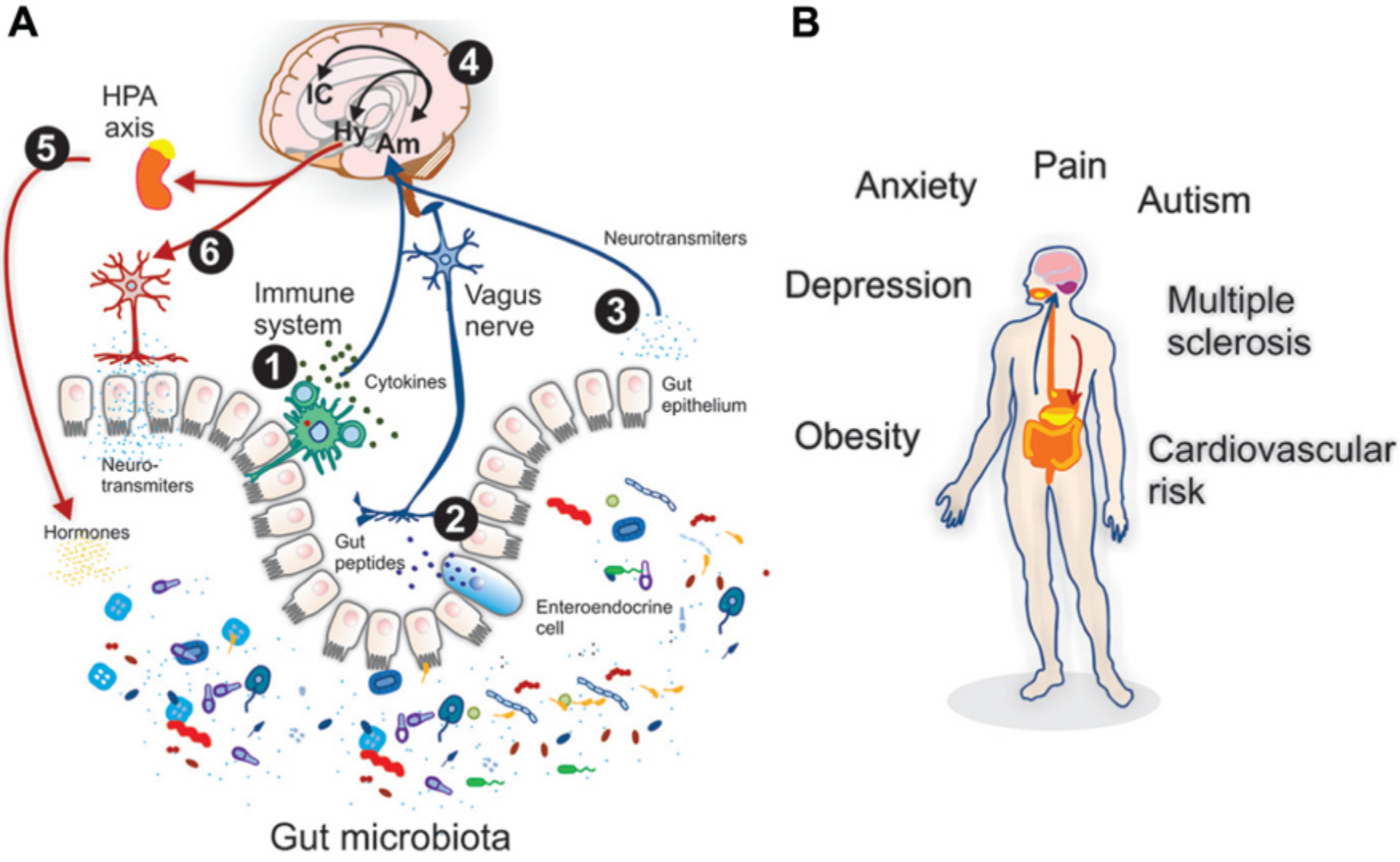
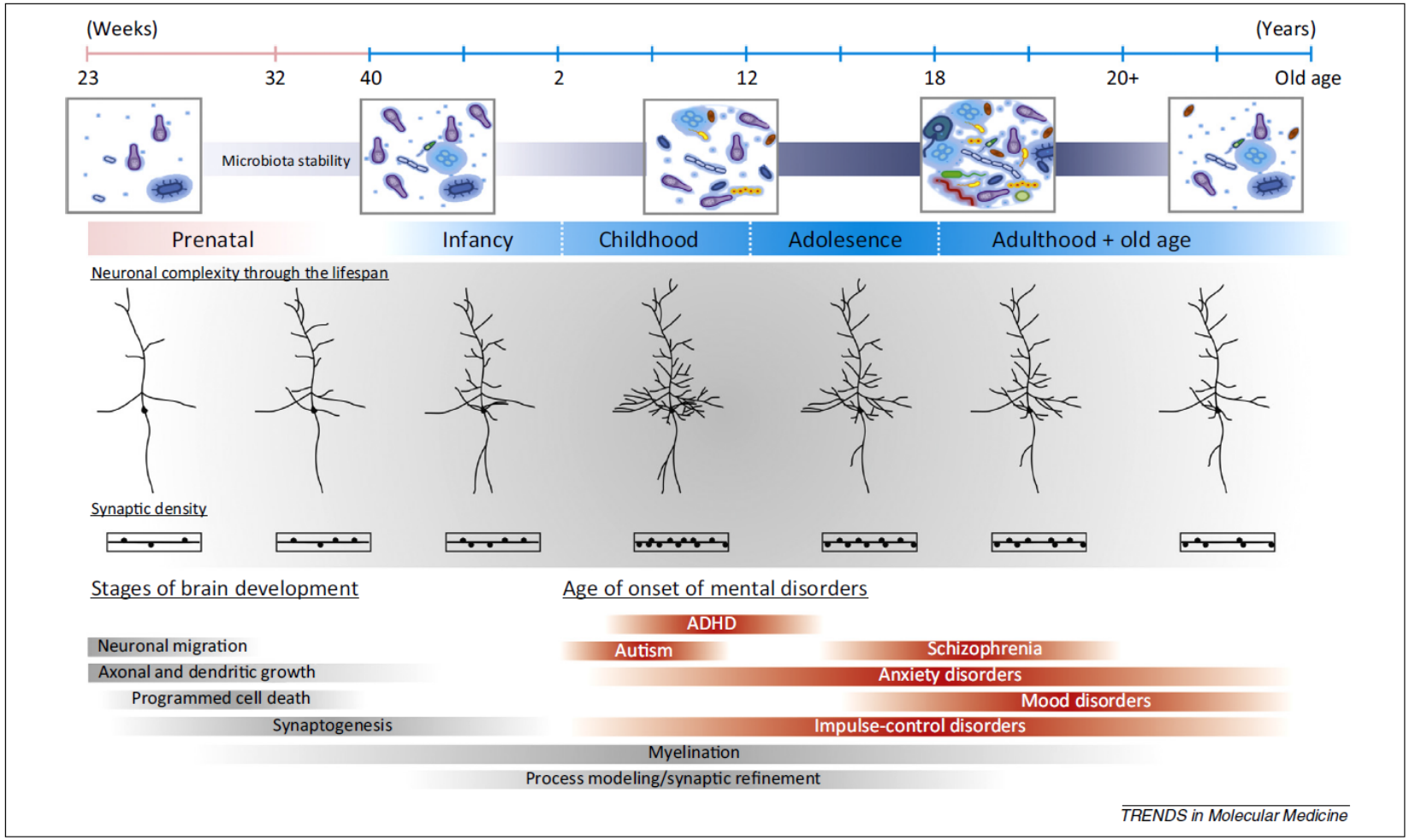
MICROBIOTA–GUT–BRAIN AXIS:



MICROBIOTA AND BRAIN DEVELOPMENT



Childhood and adolescence are critical developmental windows sensitive to damage.

Disruptions of dynamic microbiota increase the risk of (or lead to) neurodevelopmental disorders.

Review

Gut–brain axis: how the microbiome influences anxiety and depression

The Journal of Neuroscience, July 29, 2015 • 35(30):10821–10830 • 10821

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Neurobiology of Disease

Probiotics Improve Inflammation-Associated Sickness Behavior by Altering Communication between the Peripheral Immune System and the Brain

Charlotte D'Mello,[1] Natalie Ronaghan,[2] Raza Zaheer,[2] Michael Dicay,[2] Tai Le,[1] Wallace K. MacNaughton,[2] Michael G. Surrette,[3] and Mark G. Swain[1]

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Edited by Arturo Zychlinsky, Max Planck Institute fo
review August 11, 2010)

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Brain, Behavior, and Immunity

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

Altered gut microbiota and activity in a murine model of autism spectrum disorders

Caroline G.M. de Theije [b,1], Harm Wopereis [a,c,1], Mohamed Ramadan [a,b], Tiemen van Eijndthoven [a], Jolanda Lambert [a], Jan Knol [a,c], Johan Garssen [a,b], Aletta D. Kraneveld [b], Raish Oozeer [a,*]

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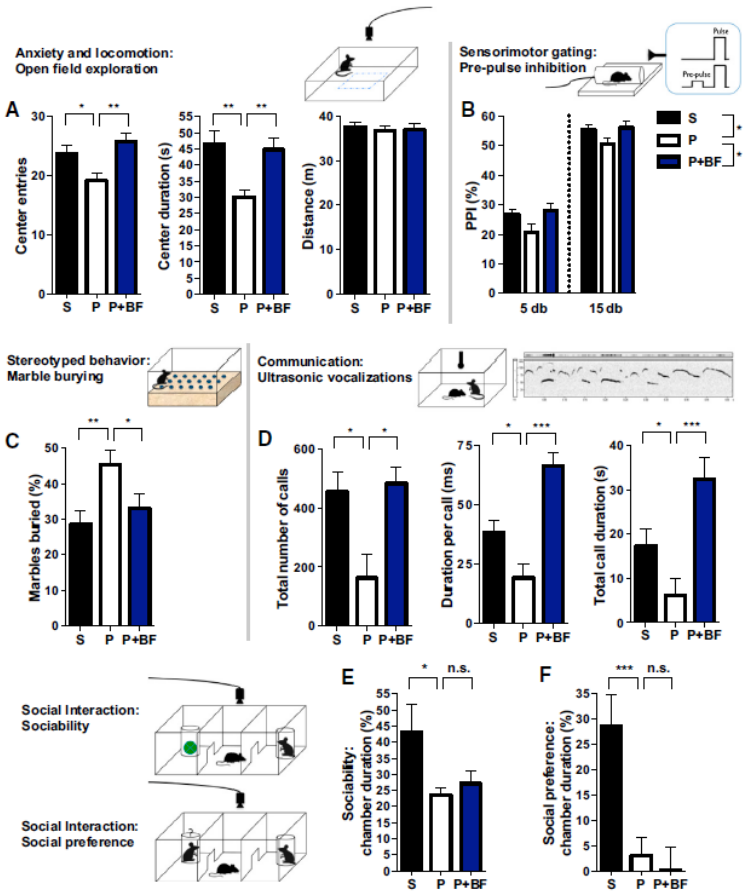
MICROBIOTA AND NEURODEVELOPMENTAL DISORDERS

B. fragilis Treatment Corrects ASD-Related Behavioral Abnormalities



Cell

Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,[1,2,*] Sara W. McBride,[1] Sophia Hsien,[1] Gil Sharon,[1] Embriette R. Hyde,[3] Tyler McCue,[3] Julian A. Codelli,[2] Janet Chow,[1] Sarah E. Reisman,[2] Joseph F. Petrosino,[3] Paul H. Patterson,[1,4,*] and Sarkis K. Mazmanian[1,4,*]
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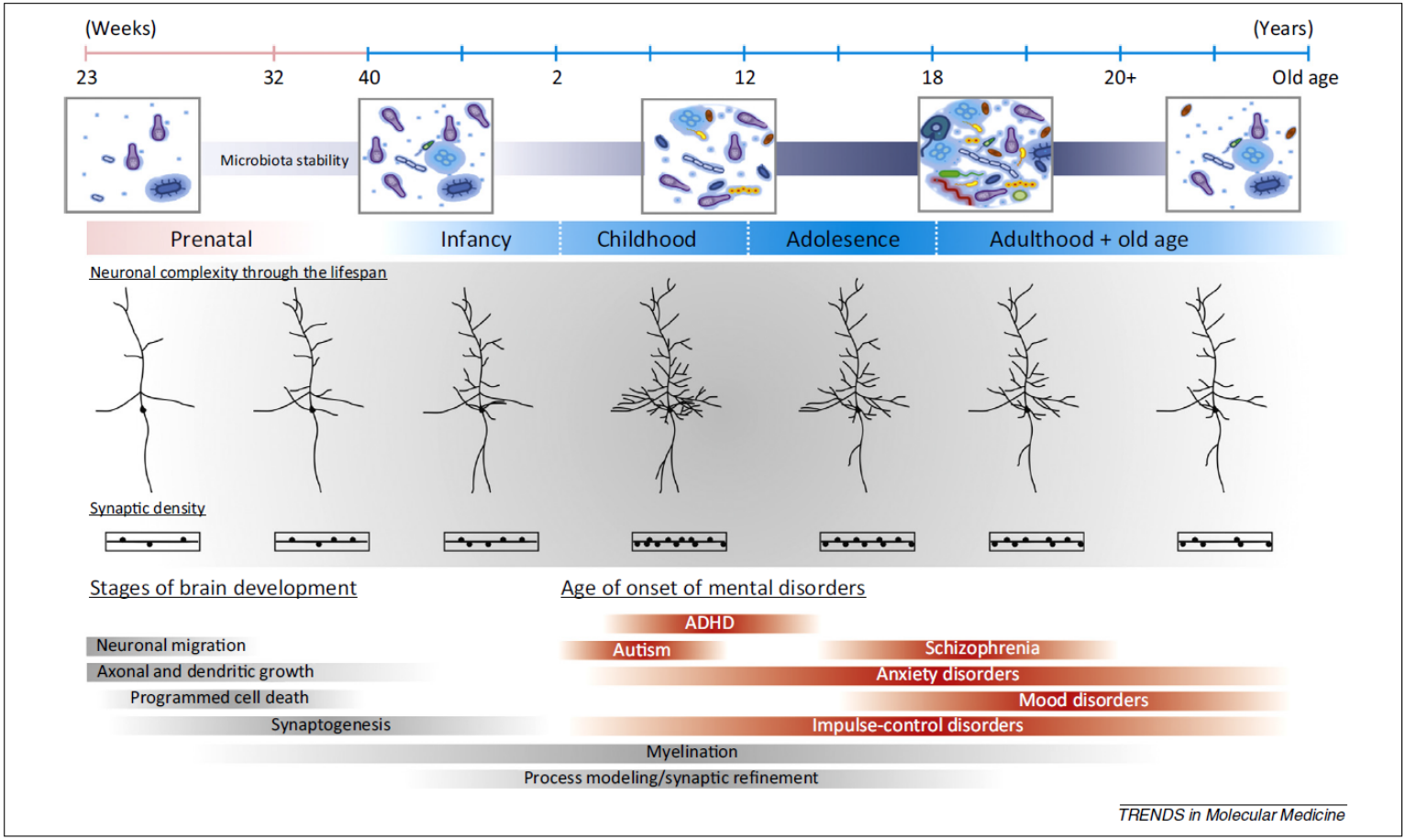
http://dx.doi.org/10.1016/j.cell.2013.11.024



Shaping of the microbiota occurs in parallel with neurodevelopment and they have similar critical developmental windows sensitive to damage.

Childhood and adolescence are the most dynamic periods of change in relation to microbiota and brain development.

Disruptions during such critical periods of dynamic microbiota–host interaction have the potential to profoundly alter brain–gut signaling, affect health throughout life, and increase the risk of (or lead to) neurodevelopmental disorders.

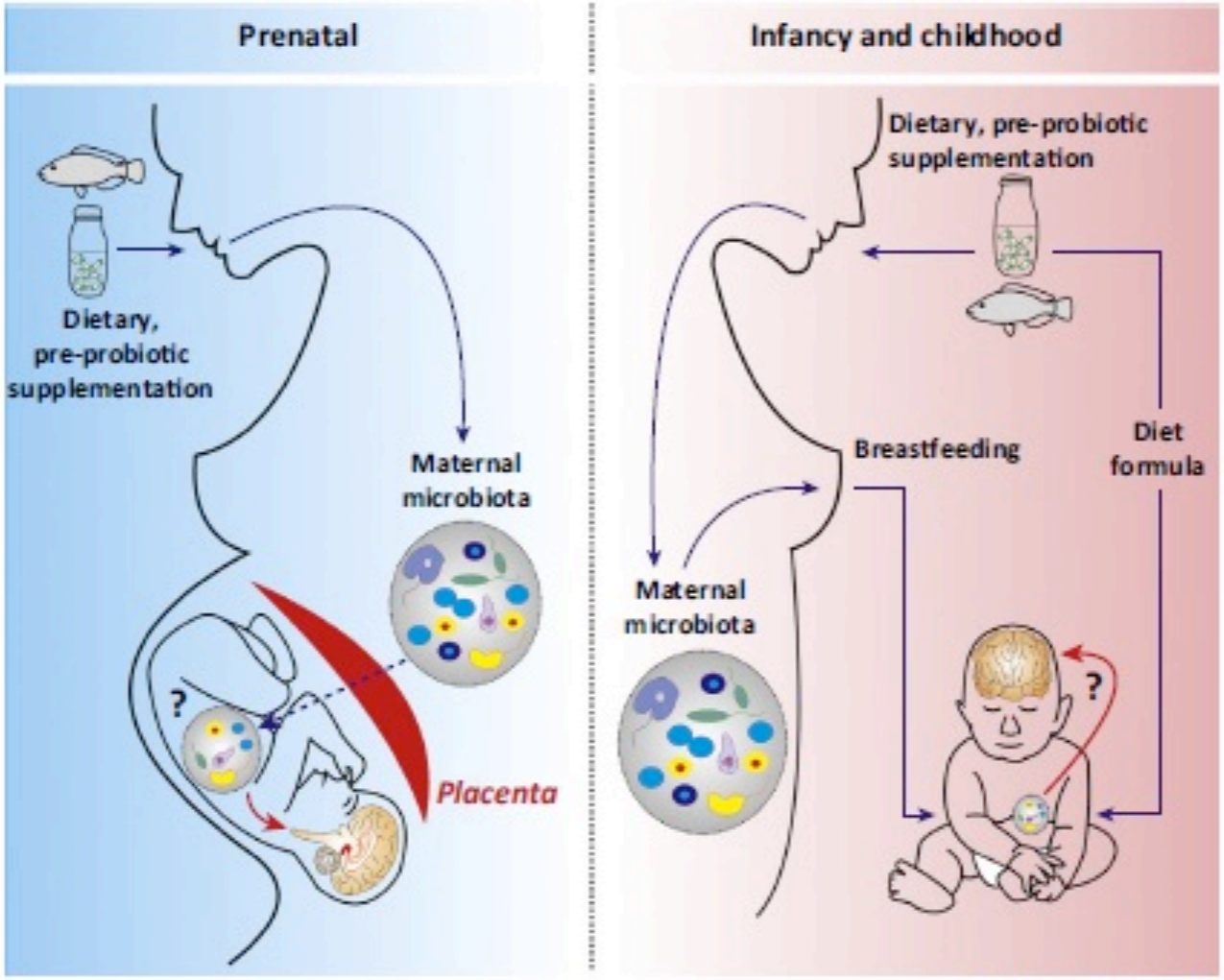


Figure 3. Windows of opportunity to modulate the microbiome of the infant prenatally and postnatally. Microbiota–gut–brain communication during prenatal and postnatal development is shown. Although still controversial, some evidence suggests that the microbiota of the infant before birth is not sterile, but may be influenced by the maternal immune state and nutrition. Prenatal and postnatal development undergoes vigorous neurodevelopmental phases and it is possible that it may be indirectly influenced by the fetal microbial population (via microbiota of the mother). This opens avenues for the development of novel dietary and microbe-modulating therapies, which may directly and indirectly alter the composition of the microbiota and neurodevelopment of the infant.

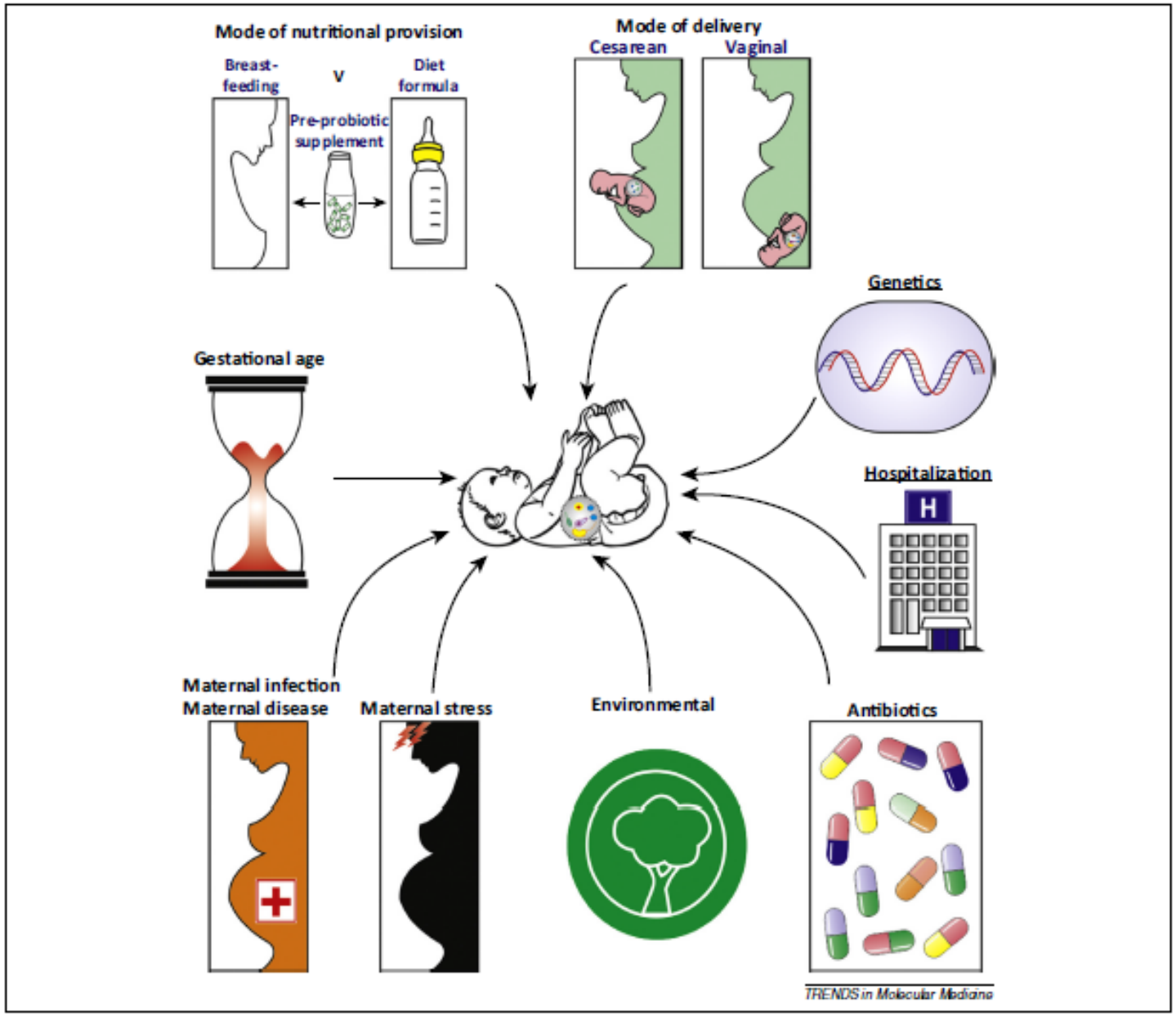
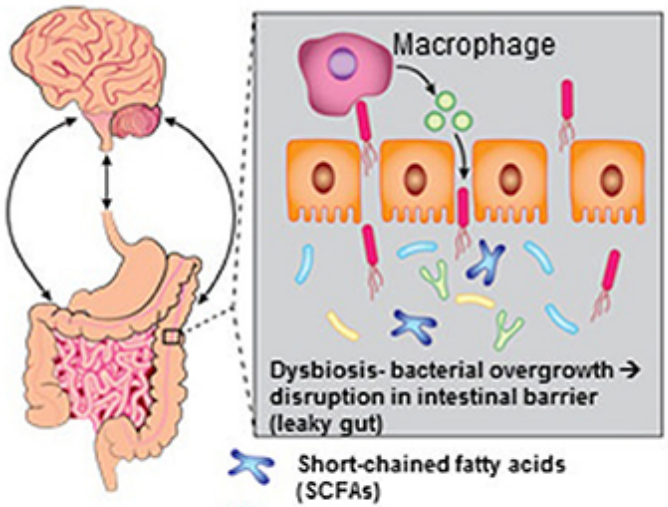
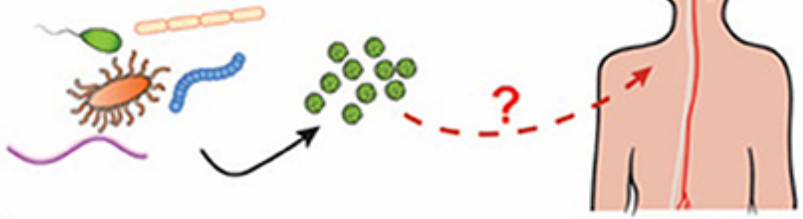


Figure 4. Factors influencing the development of the infant microbiota. Several factors play a role in shaping of the bacterial landscape in the development of the infant microbiota. In addition to mode of birth, mode of early nutrition, environment, other factors such as gestational age, genetics, and hospitalization, also influence the microbial composition of the infant. Infections (both maternal and infant) and antibiotic usage influence the trajectory of the developing microbiota as does the selective transient enrichment by probiotics and prebiotics. Taken together, such a plethora of factors with the ability to modulate the microbiota development suggest the importance of environmental influence superimposed over genetics in the establishment of a core microbiome.

Gut-Microbiome-Brain Axis

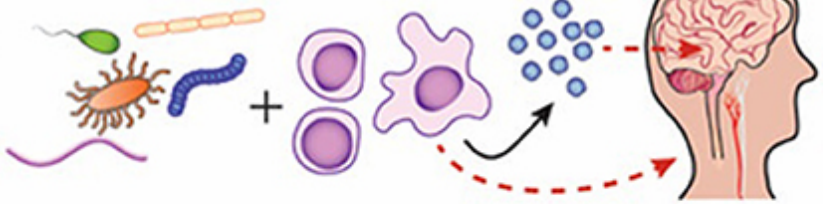


Gut microbiota production of neurotransmitters



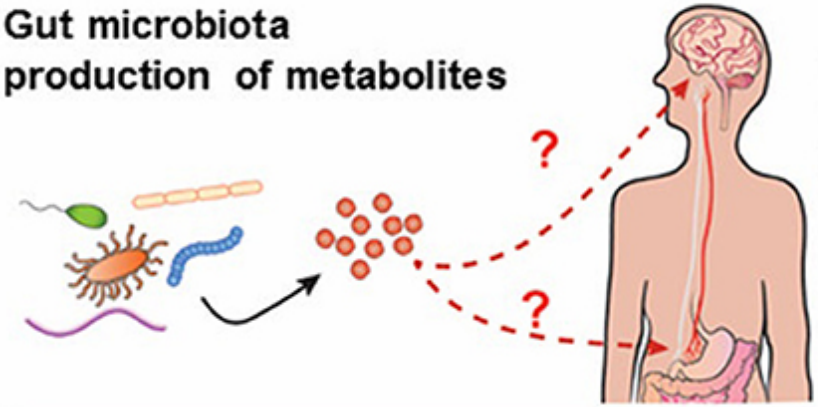
Bacterial produced neurotransmitters might travel retrograde to the brain via the vagus nerve where they can induce CNS effects

Gut microbiota may stimulate inflammatory cell production of cytokines

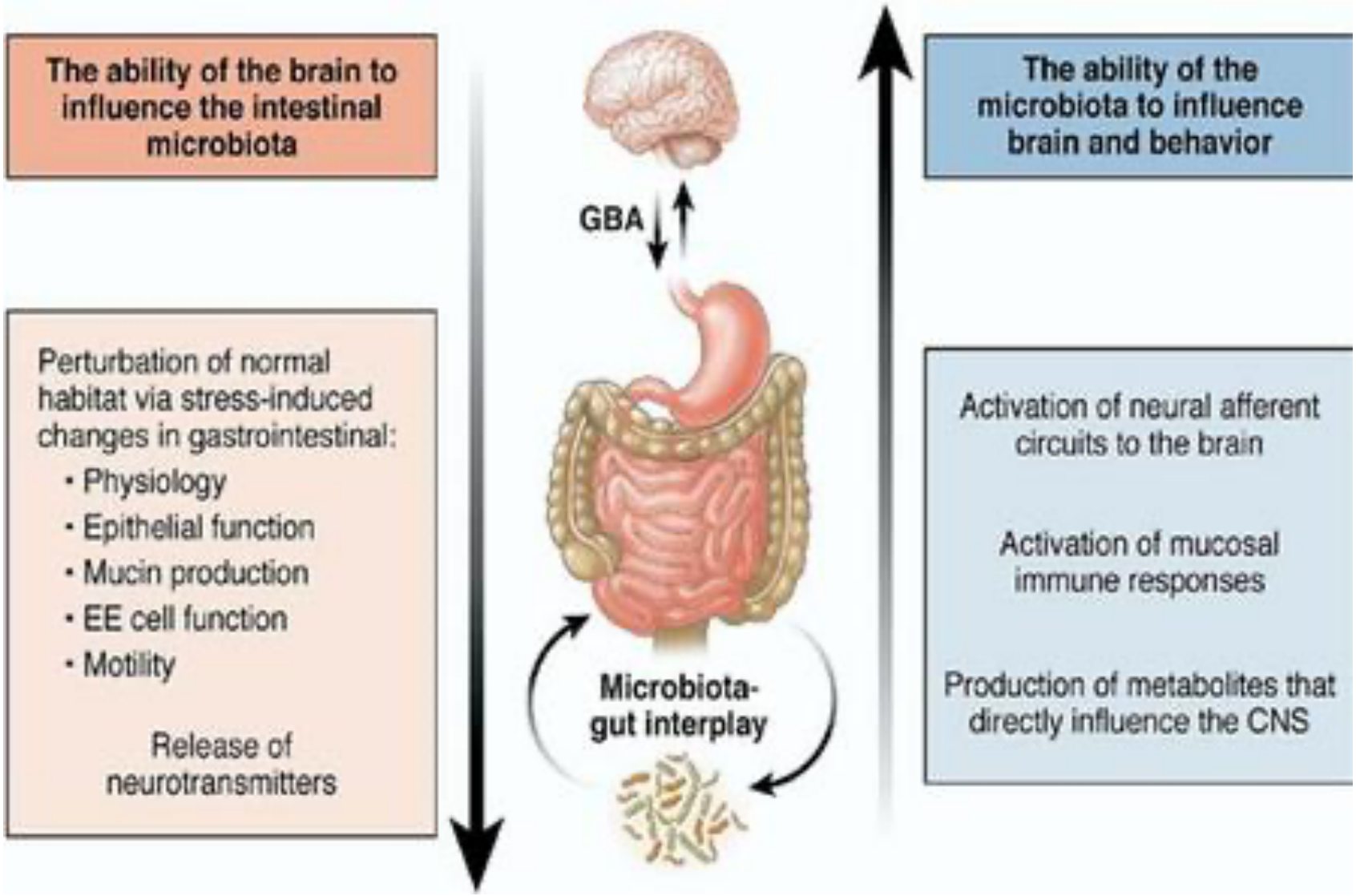


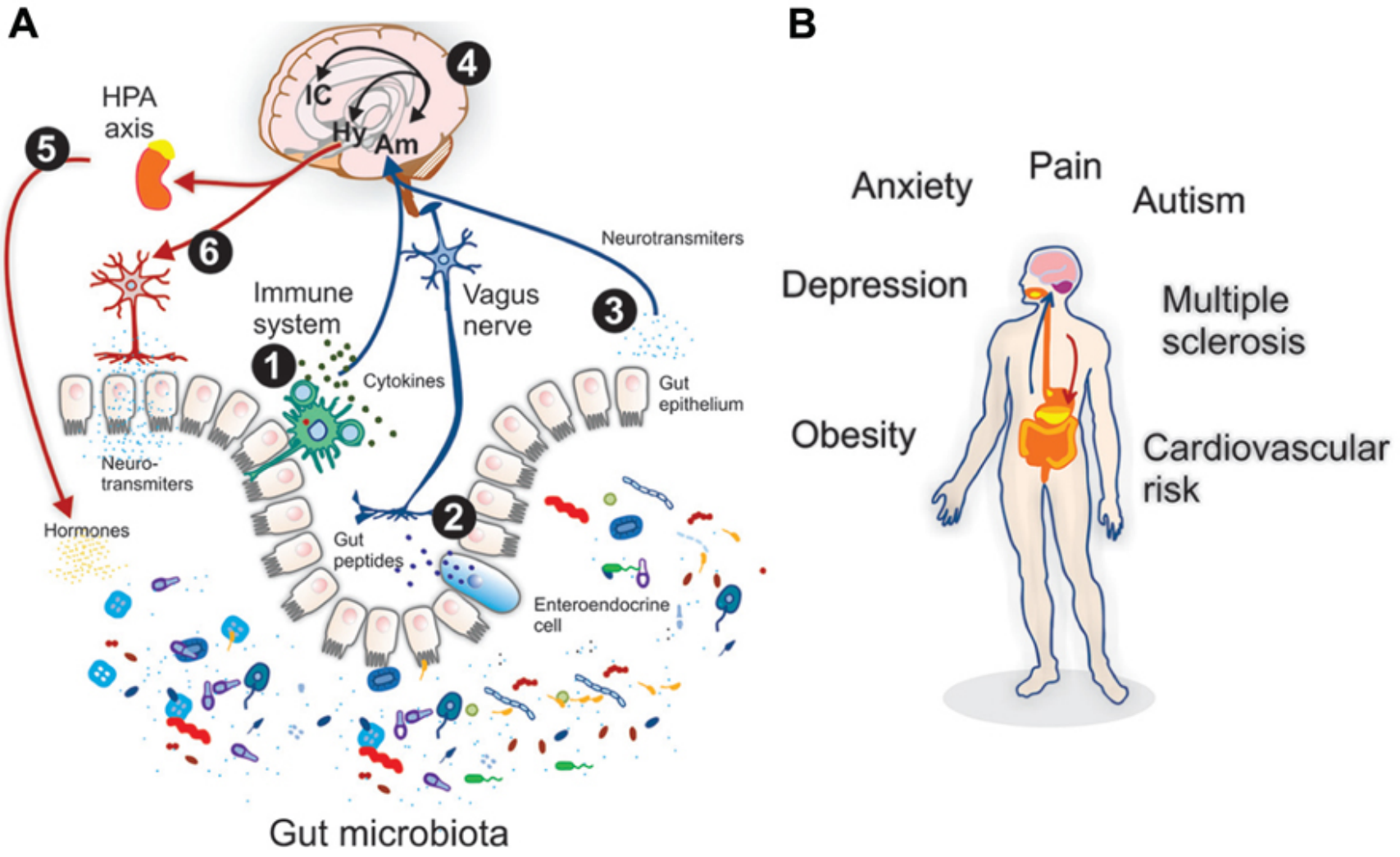
Inflammatory cytokines might travel to the brain via the systemic circulation

Gut microbiota production of metabolites



Bacterial metabolites may reach the brain. Such metabolites might also stimulate intestinal cells to produce neurotransmitters that may result in neural effects.


The ability of the brain to influence the intestinal microbiota
Perturbation of normal habitat via stress-induced changes in gastrointestinal:
• Physiology
• Epithelial function
• Mucin production
• EE cell function
• Motility
Release of neurotransmitters
GBA
Microbiota-gut interplay
The ability of the microbiota to influence brain and behavior
Activation of neural afferent circuits to the brain
Activation of mucosal immune responses
Production of metabolites that directly influence the CNS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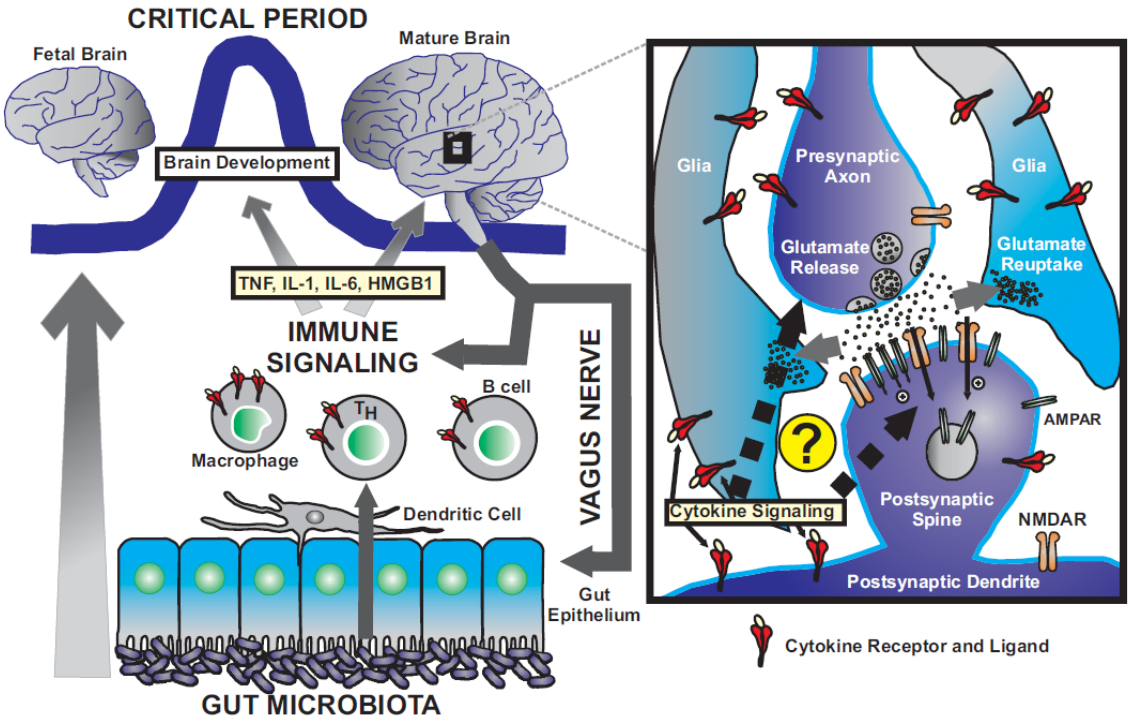
6
Immune system
1
Cytokines
Vagus nerve
Neurotransmiters
3
Gut epithelium
Neuro-transmiters
Hormones
2
Gut peptides
Enteroendocrine cell
Gut microbiota
B
Anxiety
Pain
Autism
Depression
Multiple sclerosis
Obesity
Cardiovascular risk

Both clinical and preclinical studies

Important role for the gut microbiota in the pathogenesis of ASDs,
novel therapeutic strategies in managing neurodevelopmental disorders via microbiome-based treatment.

Bacteroides fragilis given in early adolescence has been shown to ameliorate some, but not all, of the behavioral dysfunctions

The gut microbiota may be modified in throughout life and possibly pregnancy.
Early preweaning and adolescence periods appear to be critical periods for modifying enteric microbiota with the potential to prevent the development of abnormal behaviors.
Consequently, it is becoming clear that understanding the early interaction between the intestinal microbiota and the host opens novel avenues for nutritional/therapeutic interventions in at-risk populations, particularly for infants and young children.



there is a ‘‘critical period’’ that is a developmental window during which the gut flora can influence the developing brain.

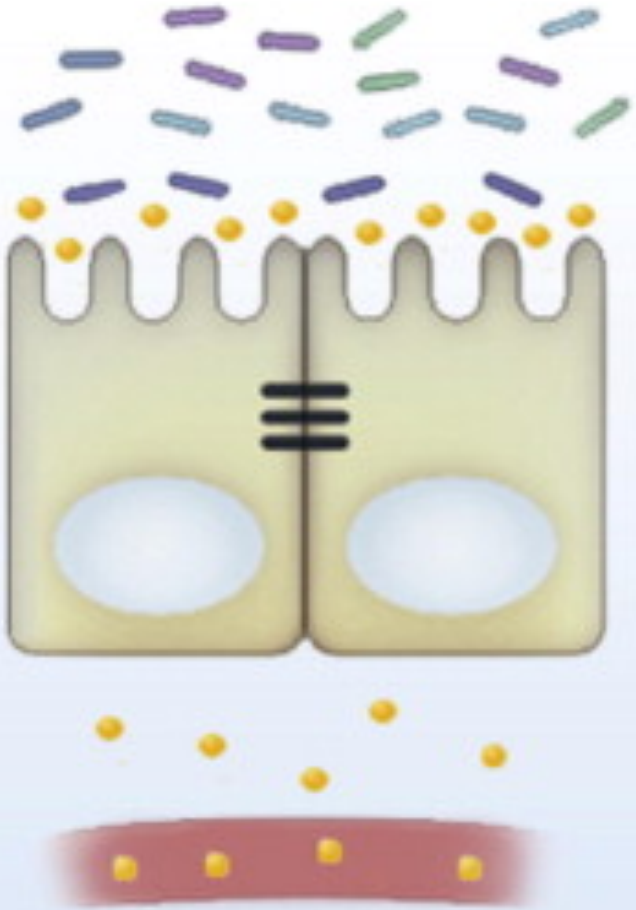
probiotic treatment of mice with autism features

alters the composition of the gut microbiota

improves epithelial barrier integrity

reduces leakage of particular GI metabolites

restores serum metabolites



ameliorates specific autism-related behavioral abnormalities

Cell

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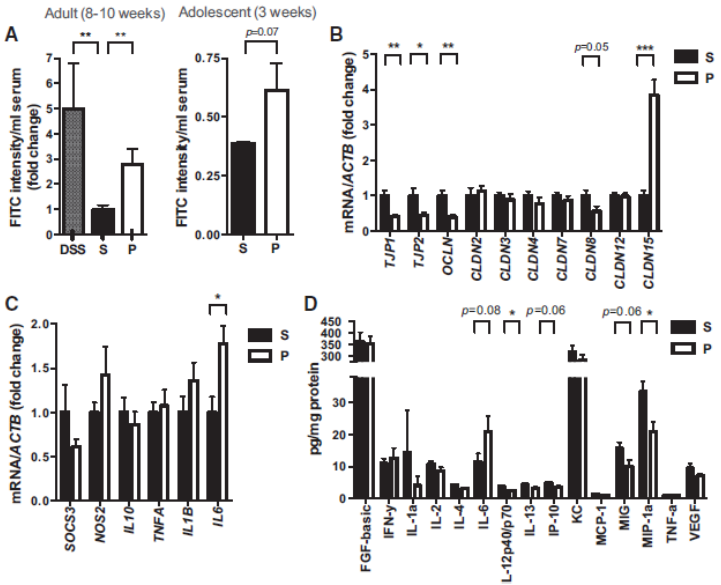
http://dx.doi.org/10.1016/j.cell.2013.11.024

Offspring of Immune-Activated Mothers Exhibit GI Symptoms of Human ASD



Animals and MIA

Pregnant C57BL/6N mice (Charles River; Wilmington, MA) were injected i.p. on E12.5 with saline or 20 mg/kg poly(I:C) according to methods described in Smith et al. (2007). All animal experiments were approved by the Caltech IACUC.

B. fragilis Treatment

Mice were selected at random for treatment with *B. fragilis* NCTC 9343 or vehicle, every other day for 6 days at weaning. 10^{10} CFU of freshly grown *B. fragilis*, or vehicle, in 1.5% sodium bicarbonate was administered in sugar-free applesauce over standard food pellets. The same procedure was used for mutant *B. fragilis* PSA and *B. thetaiotaomicron*.

Figure 1. MIA Offspring Exhibit GI Barrier Defects and Abnormal Expression of Tight Junction Components and Cytokines

(A) Intestinal permeability assay, measuring FITC intensity in serum after oral gavage of FITC-dextran. Dextran sodium sulfate (DSS): n = 6, S (saline+vehicle): adult n = 16; adolescent n = 4, P (poly(I:C)+vehicle): adult n = 17; adolescent n = 4. Data are normalized to saline controls.

(B) Colon expression of tight junction components relative to β-actin. Data for each gene are normalized to saline controls. n = 8/group.

(C) Colon expression of cytokines and inflammatory markers relative to β-actin. Data for each gene are normalized to saline controls. n = 6–21/group.

(D) Colon protein levels of cytokines and chemokines relative to total protein content. n = 10/group. For each experiment, data were collected simultaneously for poly(I:C)+*B. fragilis* treatment group (See Figure 3). See also Figure S1.

B. fragilis Treatment Corrects ASD-Related Behavioral Abnormalities

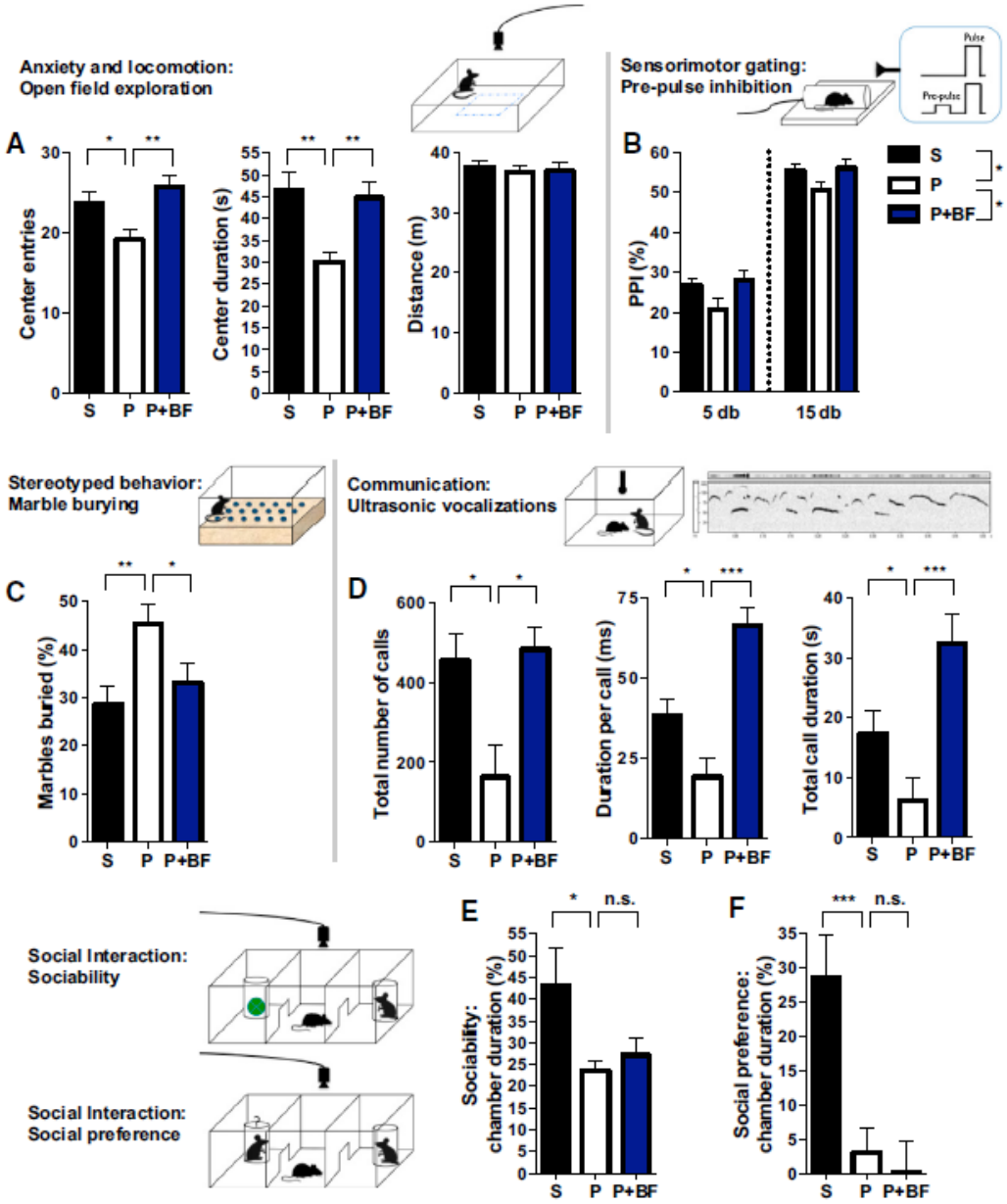


Figure 5. *B. fragilis* Treatment Ameliorates Autism-Related Behavioral Abnormalities in MIA Offspring

(A) Anxiety-like and locomotor behavior in the open field exploration assay. n = 35–75/group.

(B) Sensorimotor gating in the PPI assay. n = 35–75/group.

(C) Repetitive marble burying assay. n = 16–45/group.

(D) Ultrasonic vocalizations produced by adult male mice during social encounter. n = 10/group.

S = saline+vehicle, p = poly(I:C)+vehicle, P+BF = poly(I:C)+*B. fragilis*. Data were collected simultaneously for poly(I:C)+*B. fragilis* ΔPSA and poly(I:C)+*B. thetaiotaomicron* treatment groups (See also Figures S3 and S4).

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]

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Edited by Arturo Zychlinsky, Max Planck Institute for Infection Biology, Berlin, Germany, and accepted by the Editorial Board January 4, 2011 (received for review August 11, 2010)

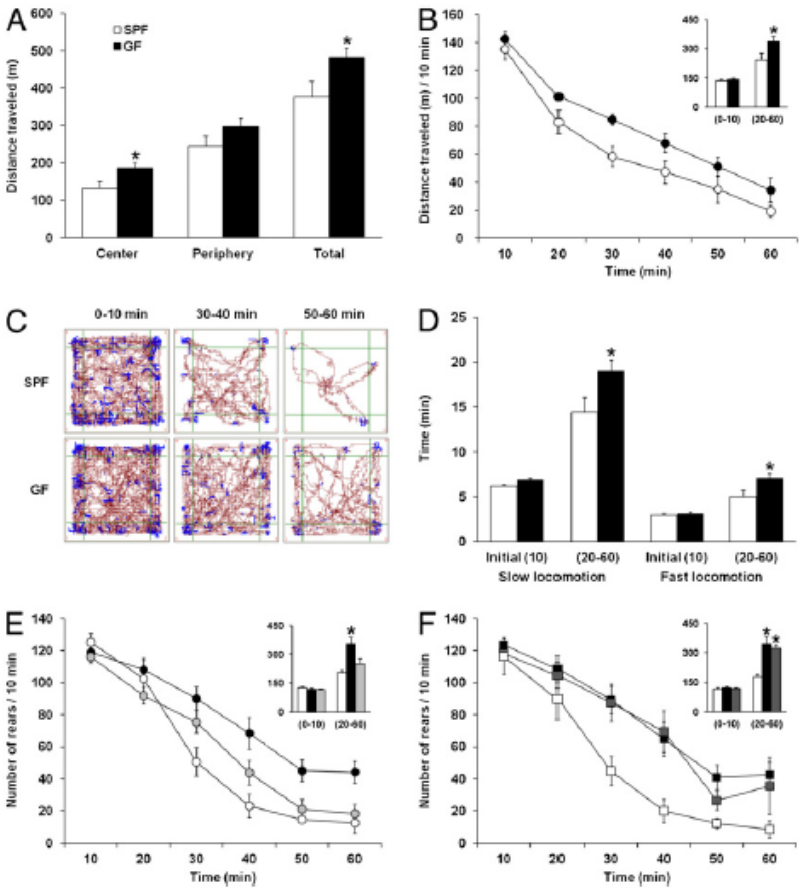


Fig. 1. GF mice display increased spontaneous motor activity. (*A*) Bars show cumulative distance traveled (meters) per zone and in the entire box (total) during the 60-min open field test session by SPF (open bars) and GF (filled bars) mice. (*B*) Average distance traveled (meters) measured in 10-min time bins across a 60-min session in an open field box. (*Inset*) Bars show cumulative distance traveled (meters) during the initial 10 min and the 20- to 60-min time interval of open field testing. (*C*) Representative tracks of movement patterns of SPF and GF mice at the 0–10, 30–40, and 50–60 min time intervals of the 60-min open field test session; distance traveled and rearing activity is shown in dark red and blue colors, respectively. (*D*) The time that SPF and GF mice spent in slow (>5 cm/s) or fast (>20 cm/s) locomotion during the initial 10 min of testing and the 20–60 min time interval. (*E*) Rearing activity of SPF (white), GF (black), and conventionalized (CON; light gray) mice. Circles show the average number of rears measured in 10-min time bins across a 60-min session in an open field box. (*F*) Rearing activity of SPF, GF, and adult CON mice (dark gray); lines connecting cumulative data in *B*, *E*, and *F* were drawn for clarity only. All data (*A*, *B*, and *D*–*F*) are presented as means (± SEM; $n = 7$–14 per group). *$P < 0.05$ compared with SPF mice.

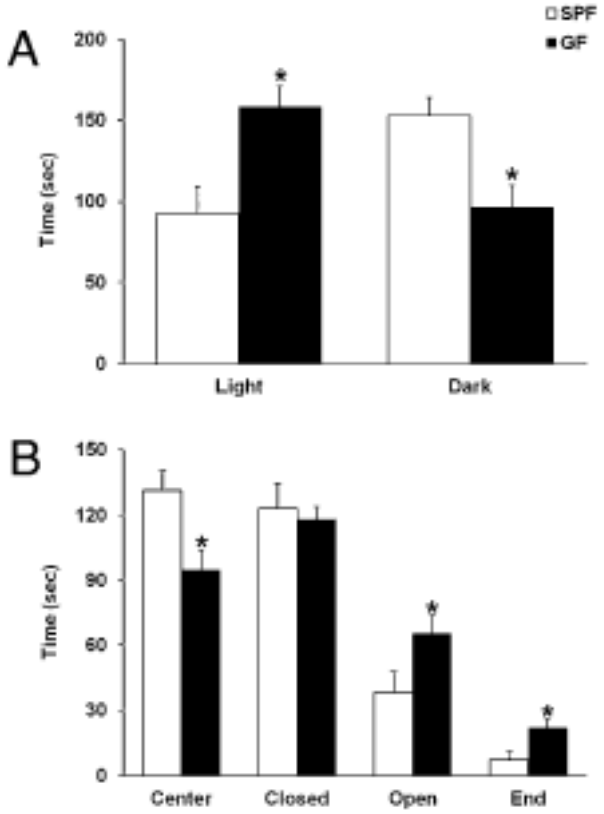


Fig. 2. GF mice display reduced anxiety-like behavior. (*A*) Bars show time (seconds) spent in the light and dark compartments during a 5-min light–dark box test by the SPF and GF mice. (*B*) Bars show time (seconds) spent in each area of the elevated plus maze by the SPF and GF mice during a 5-min test session. All data (*A* and *B*) are presented as means (±SEM; $n = 7–9$ per group). $^*P < 0.05$ compared with SPF mice.

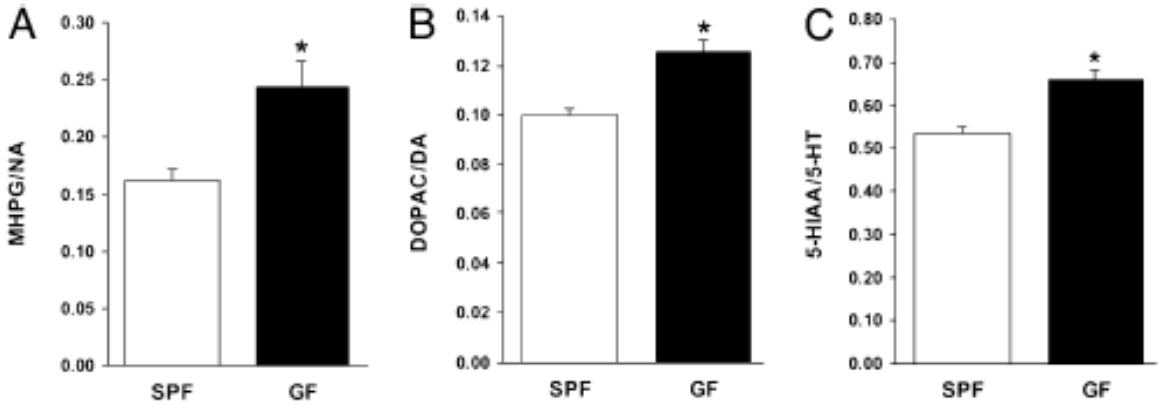


Fig. 3. GF mice show elevated NA, DA, and 5-HT turnover in the striatum. The histograms depict the mean ratios (± SEM; $n = 6$ per group) for MHPG/NA (*A*), DOPAC/DA (*B*), and 5-HIAA/5-HT (*C*) in the striatum of male GF and SPF mice. Asterisks denote where GF mice differ significantly ($P < 0.01$) from SPF mice.

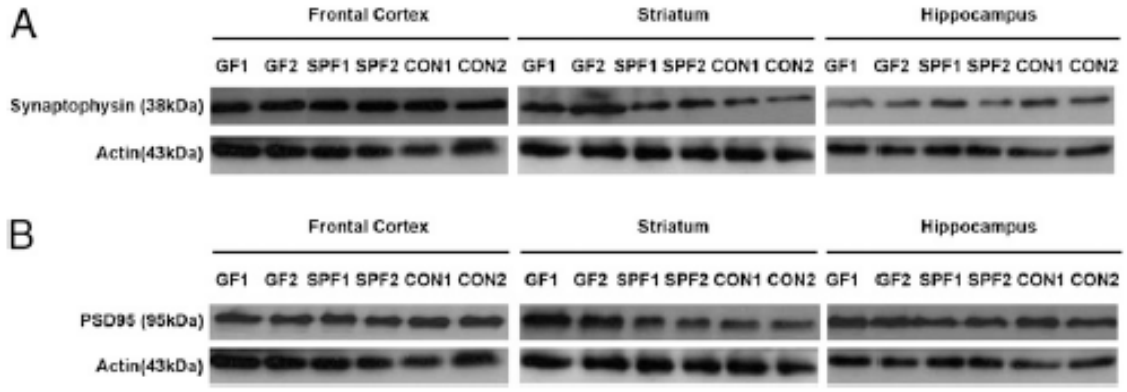
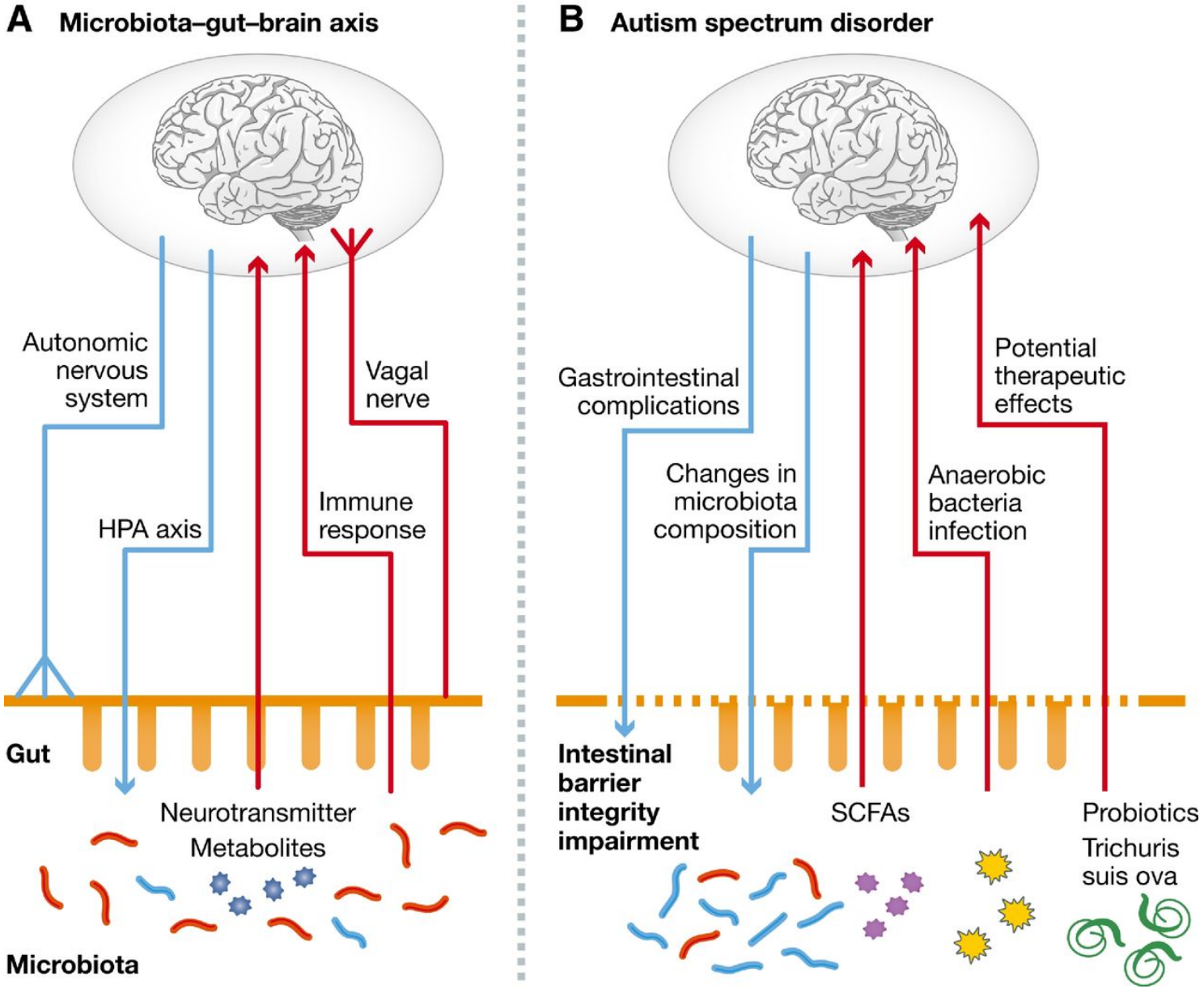
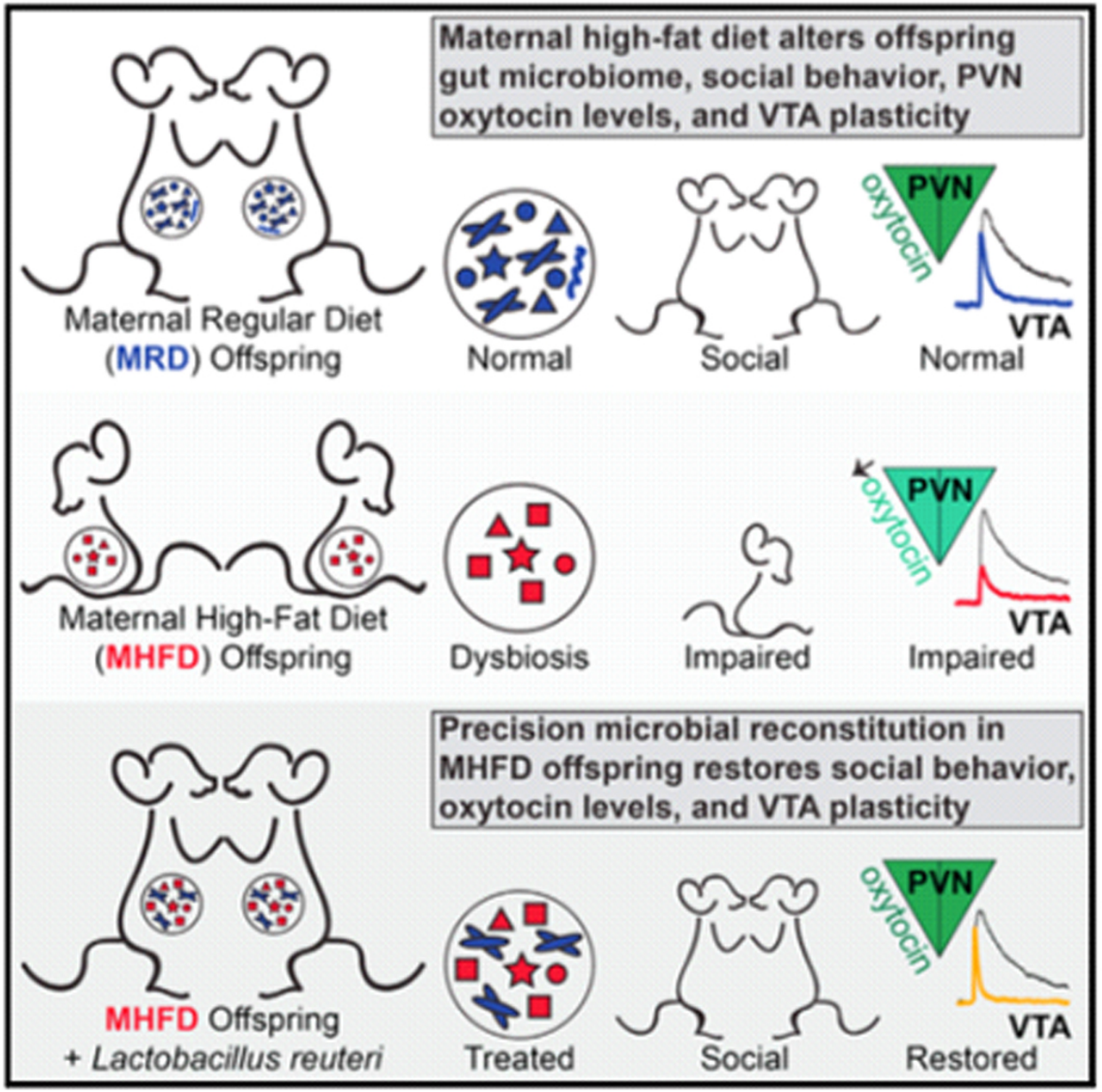


Fig. 6. GF mice show higher expression of synaptic-related proteins in the striatum compared with SPF mice. Representative Western blot films for synaptophysin (*A*) and PSD-95 (*B*) protein expression in the frontal cortex, striatum, and hippocampus of two male GF, SPF, and CON mice (for further details, see Table 1).


A Microbiota–gut–brain axis
Autonomic nervous system
Vagal nerve
HPA axis
Immune response
Gut
Neurotransmitter
Metabolites
Microbiota
B Autism spectrum disorder
Gastrointestinal complications
Potential therapeutic effects
Changes in microbiota composition
Anaerobic bacteria infection
Intestinal barrier integrity impairment
SCFAs
Probiotics
Trichuris suis ova

RESCUE?



CREDIT: BUFFINGTON ET AL./CELL 2016

Fig 6- www.deviantart.com

Schizophrenia

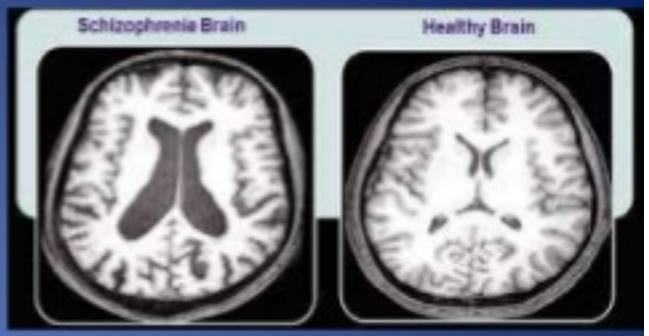


Fig 7-www.hindustanlink.com

- Lack of microbiota and elevated pro-inflammatory cytokines is seen in schizophrenic patients compared to controls. (Francesconi et al., 2011, and Song et al., 2013)

- Side effects associated with Schizophrenia such as metabolic syndrome and autoimmune disorders could be attributed to changes in microbiota. However no theories are proven.

Adult Hippocampal Neurogenesis Is Regulated by the Microbiome

To the Editor:

Biological Psychiatry

Correspondence

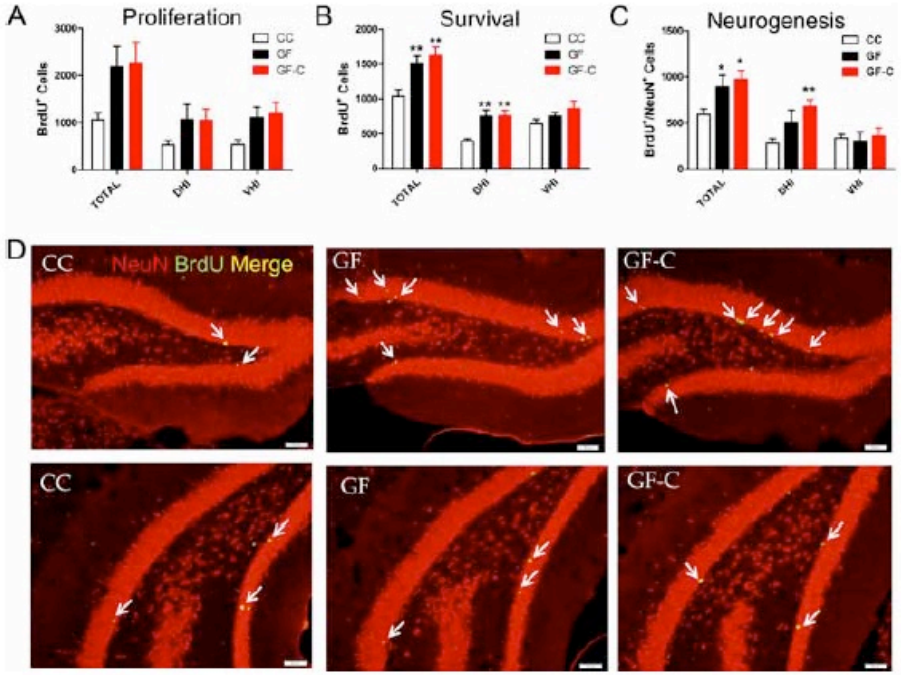
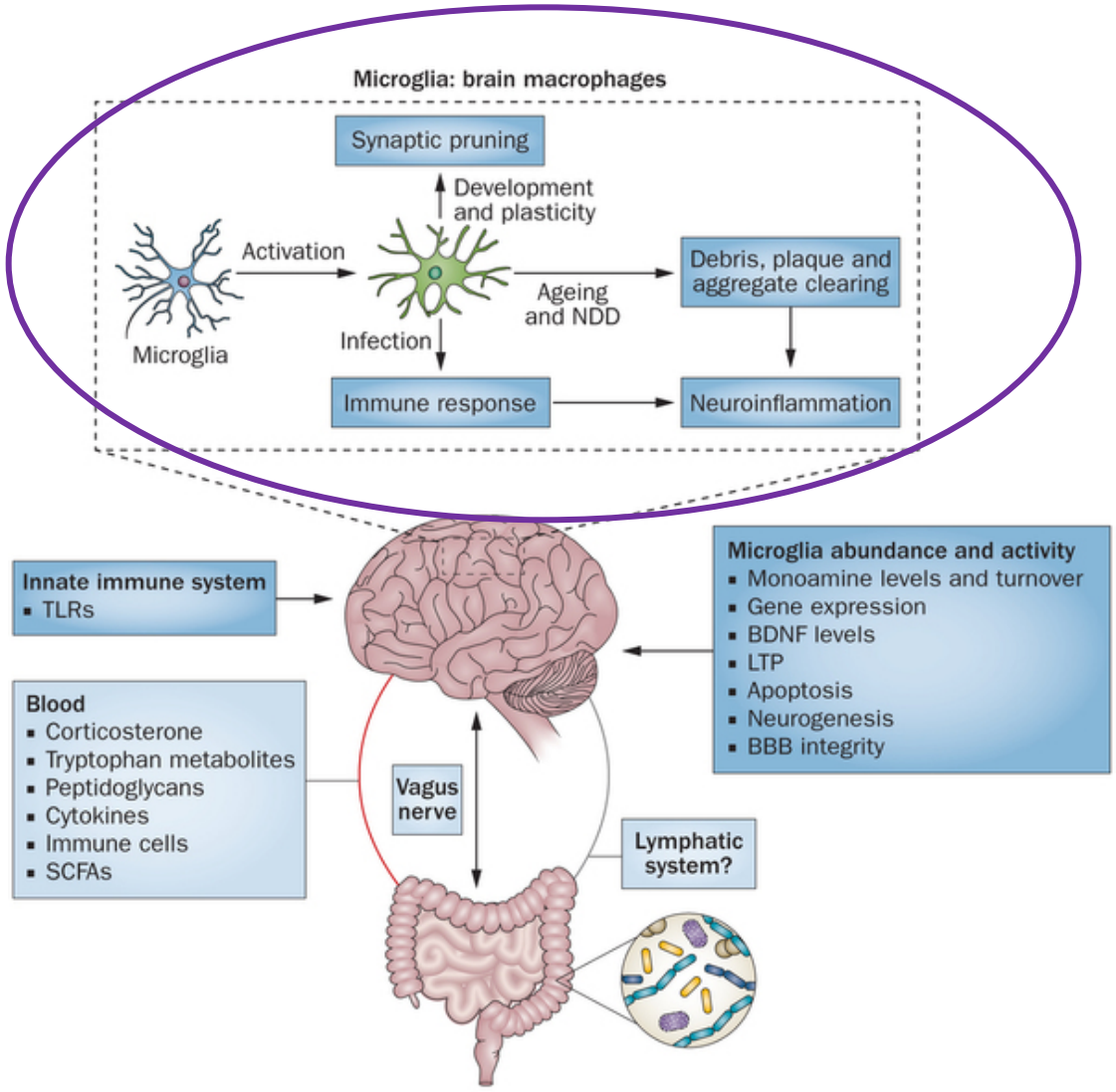


Figure 1. Germ-free mice exhibit increased adult hippocampal neurogenesis. Germ-free and germ-free–colonized mice exhibit a trend for increased cell proliferation as measured by bromodeoxyuridine immunohistochemistry **(A)**. The survival of newly born cells is significantly increased in the dorsal, but not ventral, hippocampus of germ-free and germ-free–colonized mice **(B)**. The survival of newly born neurons is increased in germ-free and germ-free–colonized mice **(C)**, and this effect occurs preferentially in the dorsal hippocampus **(C, D**—upper panels) and not the ventral hippocampus **(C, D**—lower panels). $^{*}p < .05$, $^{**}p < .01$ significantly different from conventionally colonized control mice. BrdU, bromodeoxyuridine; CC, conventionally colonized; DHI, dorsal hippocampus; GF, germ-free; GF-C, germ-free colonized; NeuN, neuronal nucleus; VHI, ventral hippocampus.

least significant difference post hoc test for group-wise comparisons.

Across the total SGZ, cell proliferation (Figure 1A) was increased in GF and GF-C mice, although the effect did not

THE MICROGLIAL SIDE OF THE MICROBIOTA–GUT–BRAIN AXIS



Nature Reviews | Gastroenterology & Hepatology

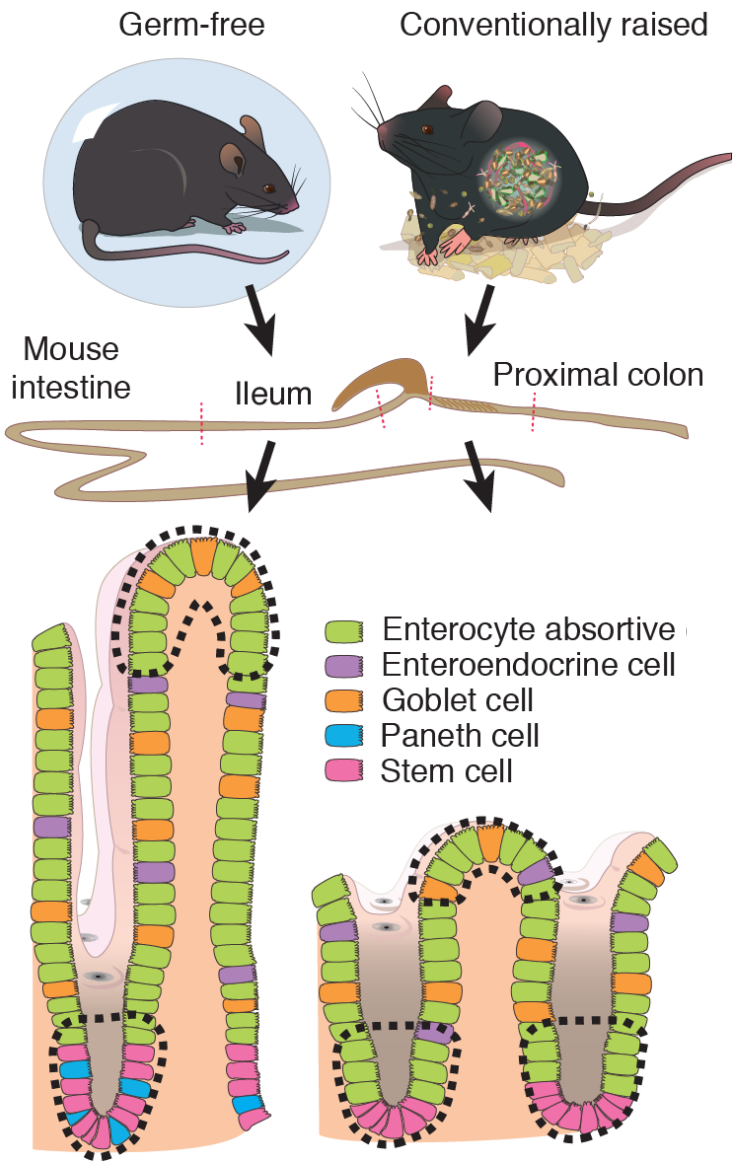
ARTICLES

nature neuroscience

Host microbiota constantly control maturation and function of microglia in the CNS

Daniel Erny[1,12], Anna Lena Hrabě de Angelis[1,12], Diego Jaitin[2], Peter Wieghofer[1,3], Ori Staszewski[1], Eyal David[2], Hadas Keren-Shaul[2], Tanel Mahlakoiv[4], Kristin Jakobshagen[5], Thorsten Buch[6], Vera Schwierzeck[7], Olaf Utermöhlen[5], Eunyoung Chun[8], Wendy S Garrett[8], Kathy D McCoy[9], Andreas Diefenbach[7], Peter Staeheli[4], Bärbel Stecher[10], Ido Amit[2] & Marco Prinz[1,11]

THE MICROGLIAL SIDE OF THE MICROBIOTA–GUT–BRAIN AXIS



GF animals display global defects in microglia:

- Increased expression of maturation and activation marker in GF microglia.
- M1- and M2-related genes were only marginally changed, whereas most differently regulated genes were found to localize in the M0 cluster, indicating that microglia steady-state condition was severely altered in the absence of microbiota.

nature neuroscience ARTICLES

Host microbiota constantly control maturation and function of microglia in the CNS

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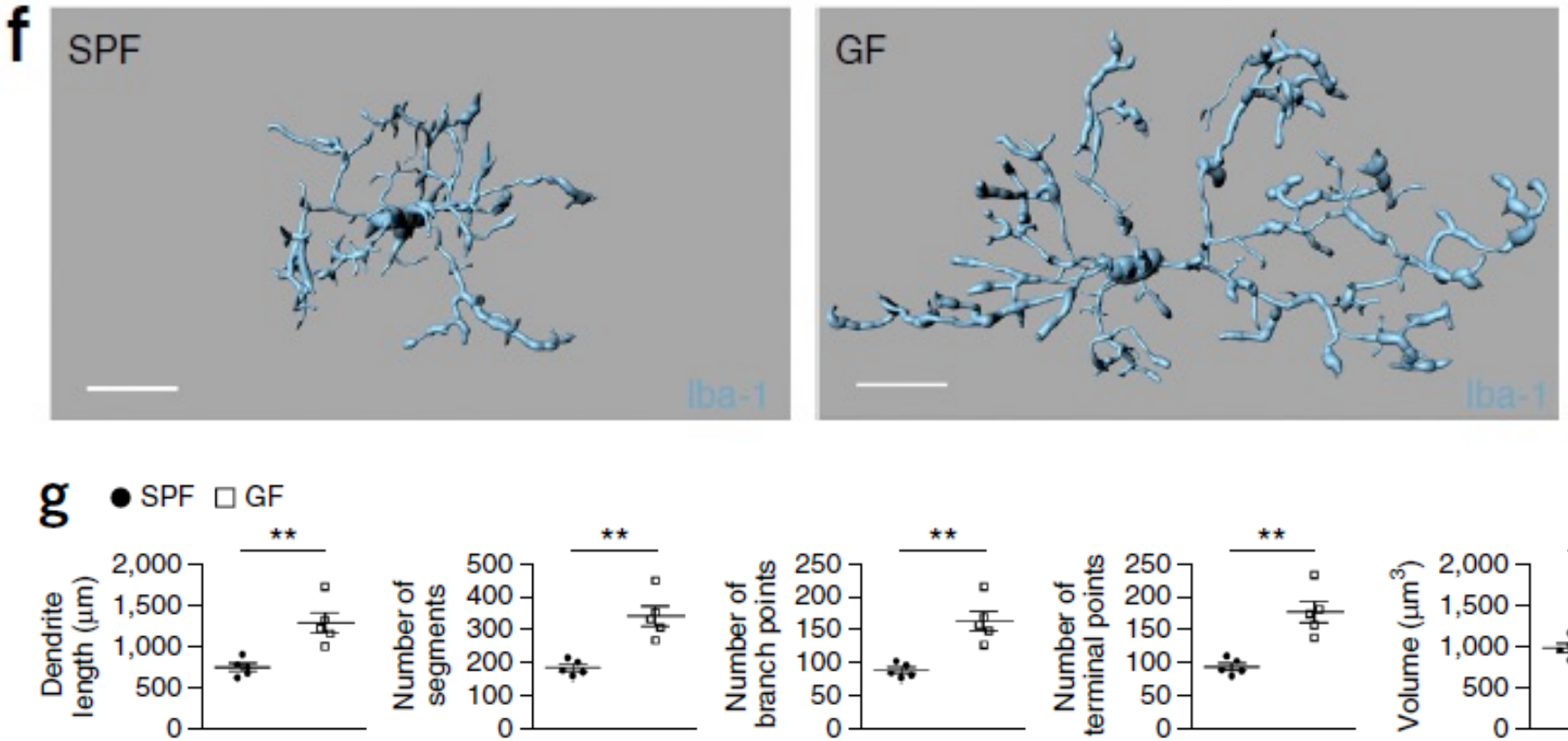
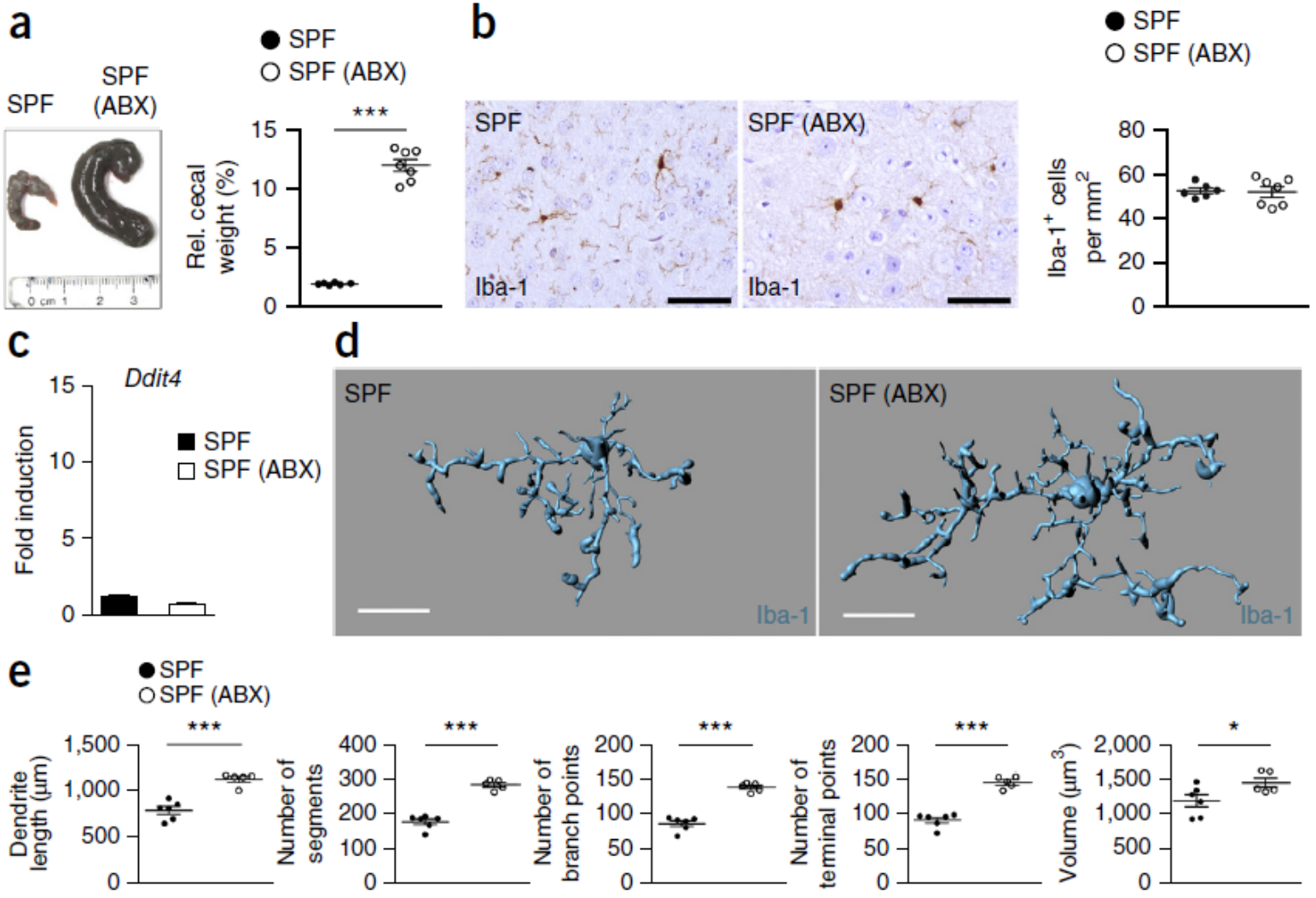
LACK OF MICROBES IMPAIRS MICROGLIA MORPHOLOGY

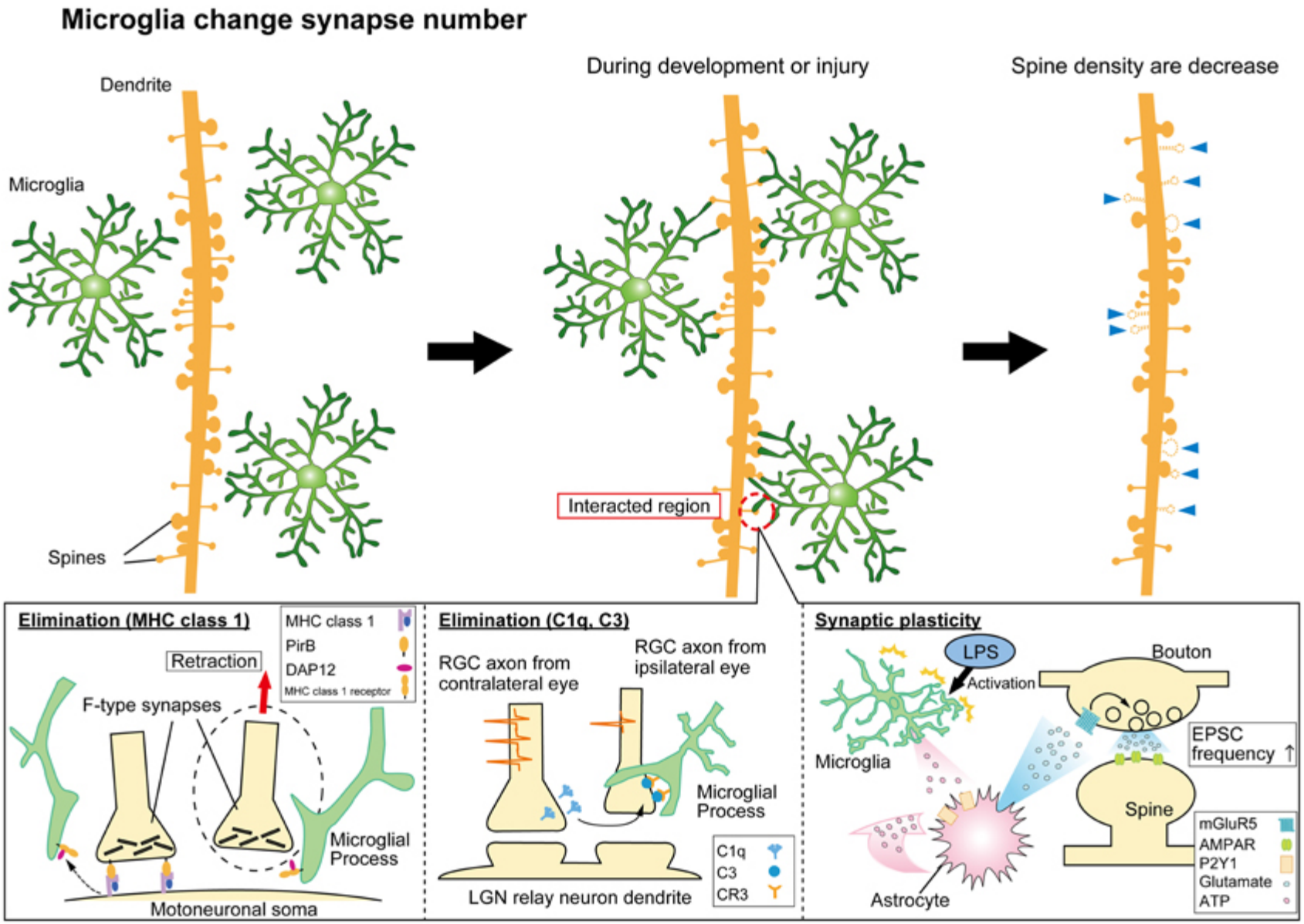


Increased microglia cell numbers with significantly longer processes and increased numbers of segments, branching and terminal points.

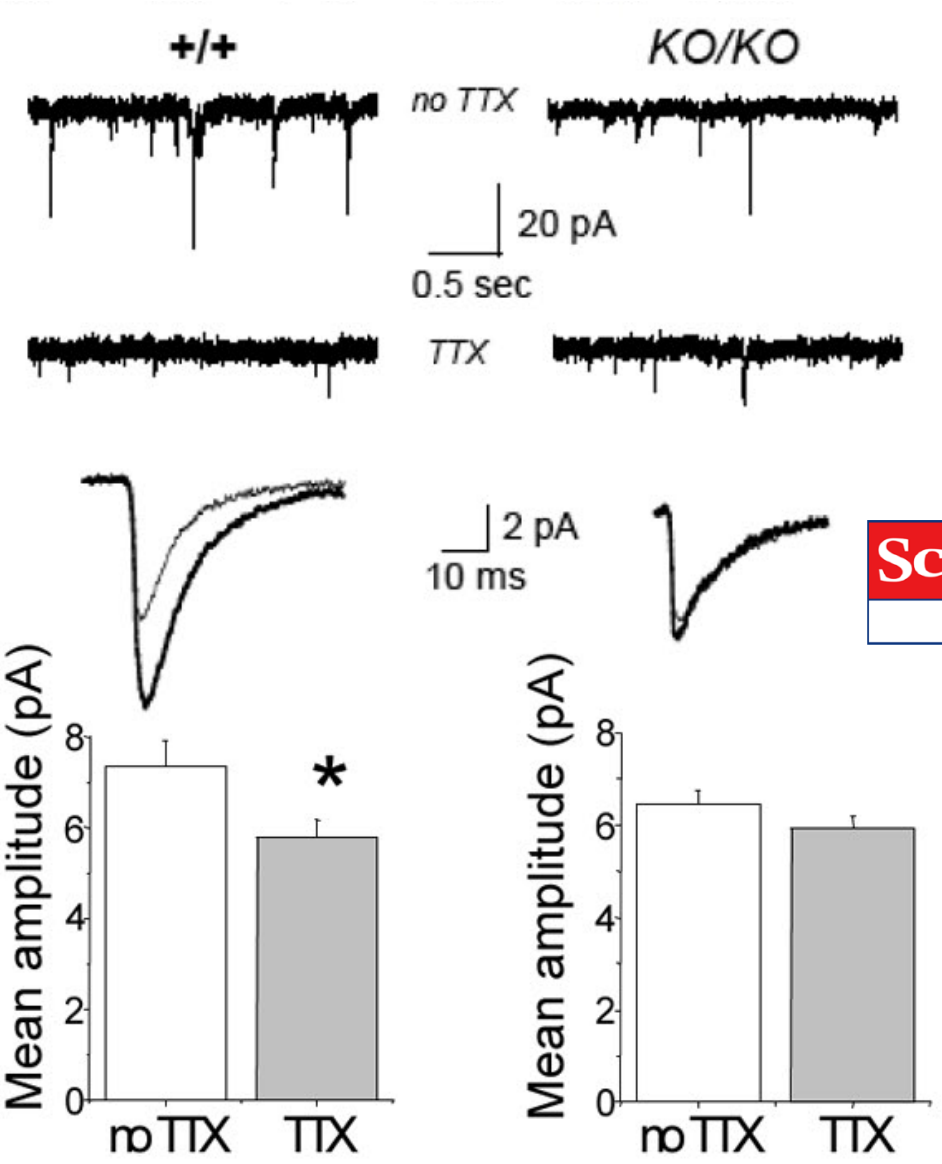
ANTIBIOTIC TREATMENT INDUCES IMMATURE AND MALFORMED MICROGLIA THAT CAN BE RESTORED BY SCFA ADMINISTRATION



MICROGLIAL CONTROL OF SYNAPTIC DEVELOPMENT



DEVELOPING CX3CR1 KO MICE DISPLAY IMMATURE SYNAPTIC FEATURES

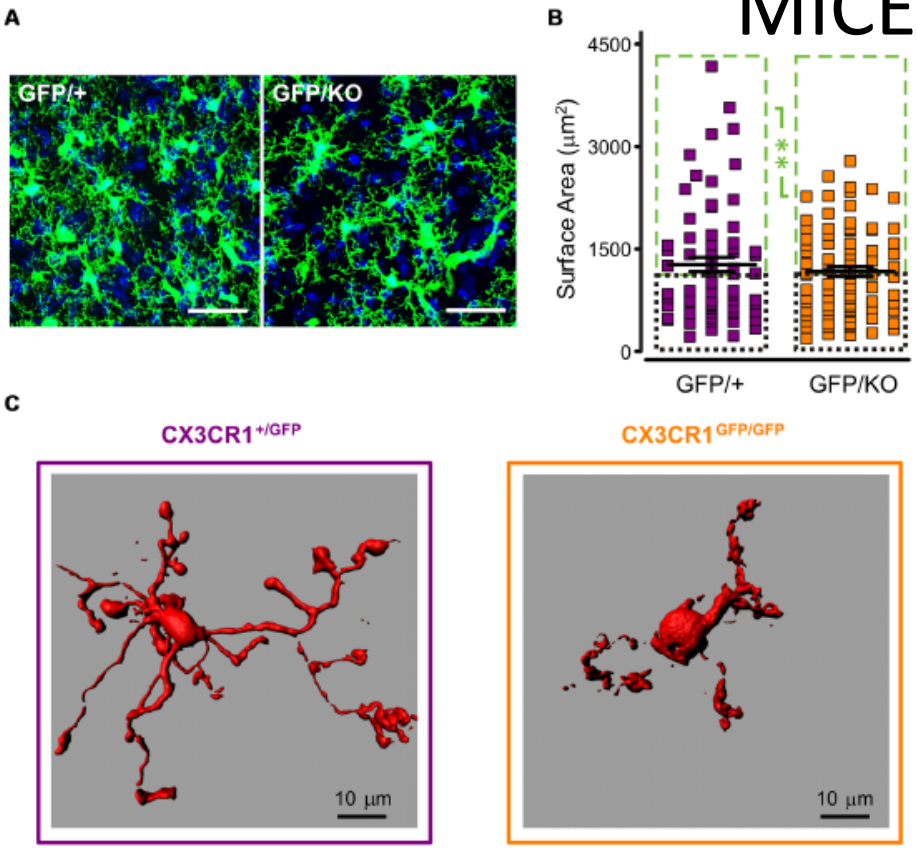


REPORTS

Synaptic Pruning by Microglia Is Necessary for Normal Brain Development

Rosa C. Paolicelli,[1] Giulia Bolasco,[1] Francesca Pagani,[2] Laura Maggi,[2] Maria Scianni,[2] Patrizia Panzanelli,[3] Maurizio Giustetto,[3,4] Tiago Alves Ferreira,[1] Eva Guiducci,[1] Laura Dumas,[1] Davide Ragozzino,[2] Cornelius T. Gross[1]*

DEFECTIVE MICROGLIAL DEVELOPMENT IN CX3CR1 KO MICE



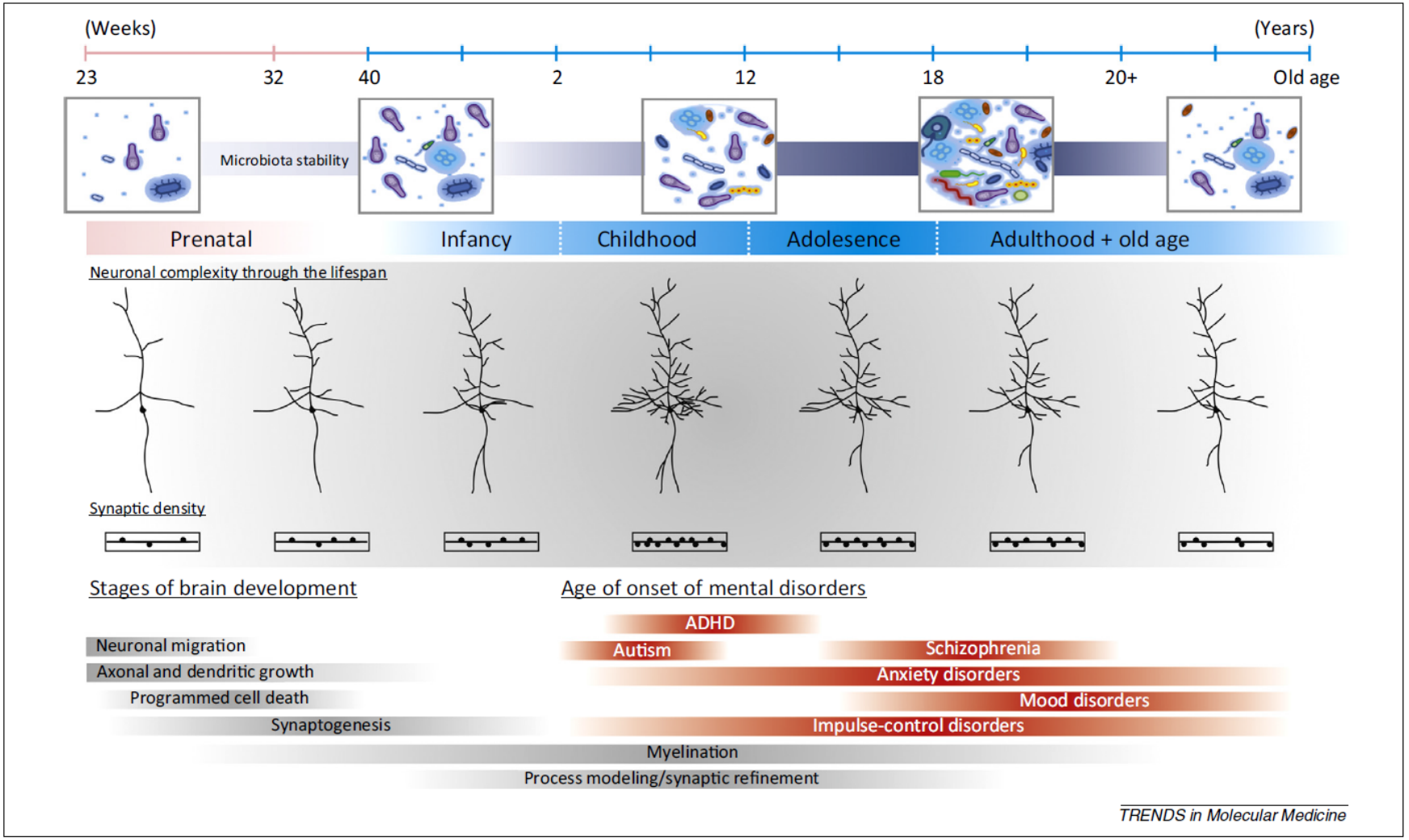
frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 31 March 2015
doi: 10.3389/fncel.2015.00111

Defective microglial development in the hippocampus of *Cx3cr1* deficient mice

Francesca Pagani[1†], Rosa C. Paolicelli[2,3†], Emanuele Murana[4], Barbara Cortese[5], Silvia Di Angelantonio[1,4], Emanuele Zurolo[6], Eva Guiducci[3], Tiago A. Ferreira[3], Stefano Garofalo[4], Myriam Catalano[4,7], Giuseppina D'Alessandro[4,7], Alessandra Porzia[3], Giovanna Peruzzi[1], Fabrizio Mainiero[5], Cristina Limatola[4,7], Cornelius T. Gross[3] and Davide Ragozzino[4,7]*

MICROBIOTA AND BRAIN DEVELOPMENT



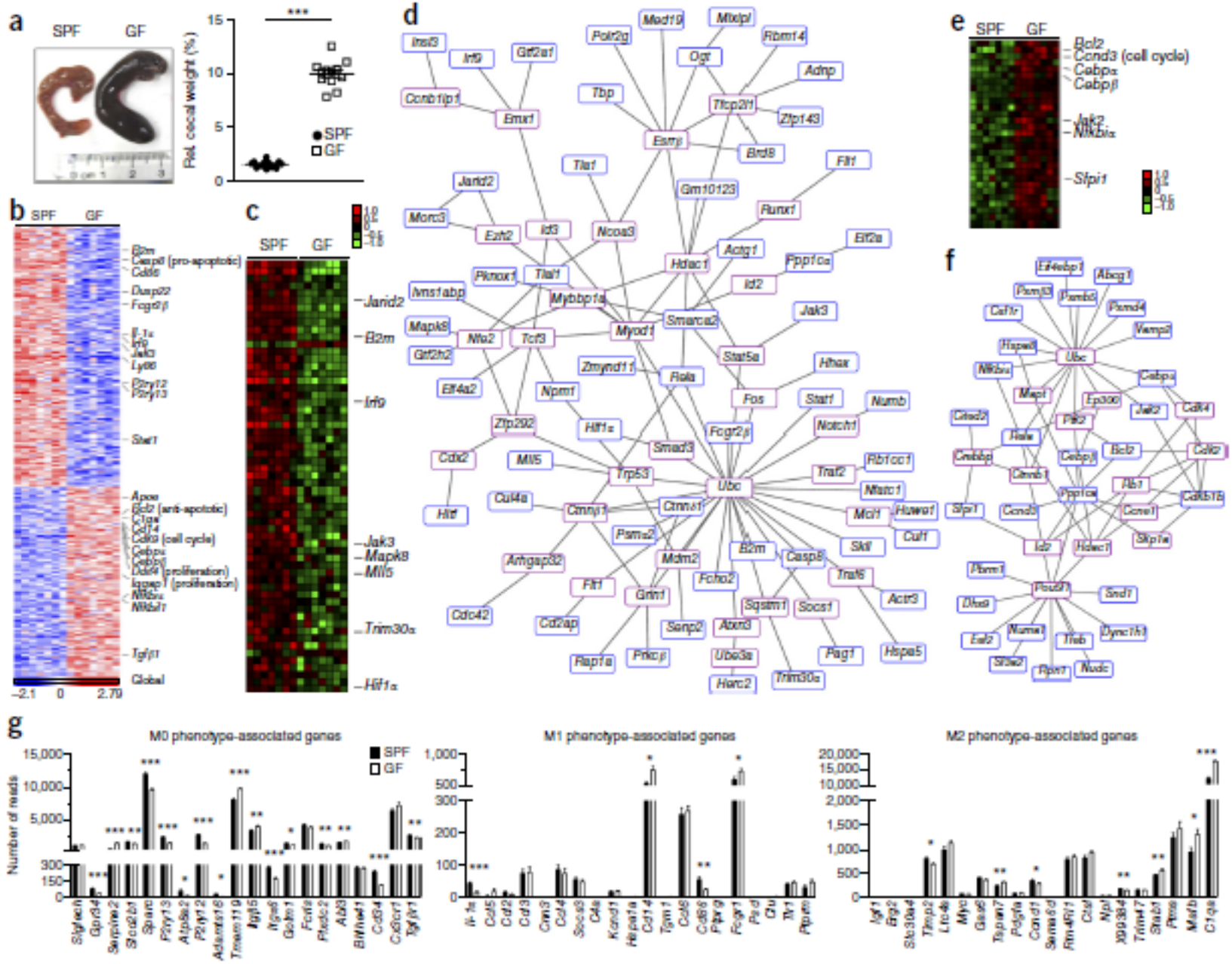


Figure 1 Altered microglial gene profile and immaturity in GF animals. (**a**) Left, photograph of caeca from SPF (control) and GF mice.

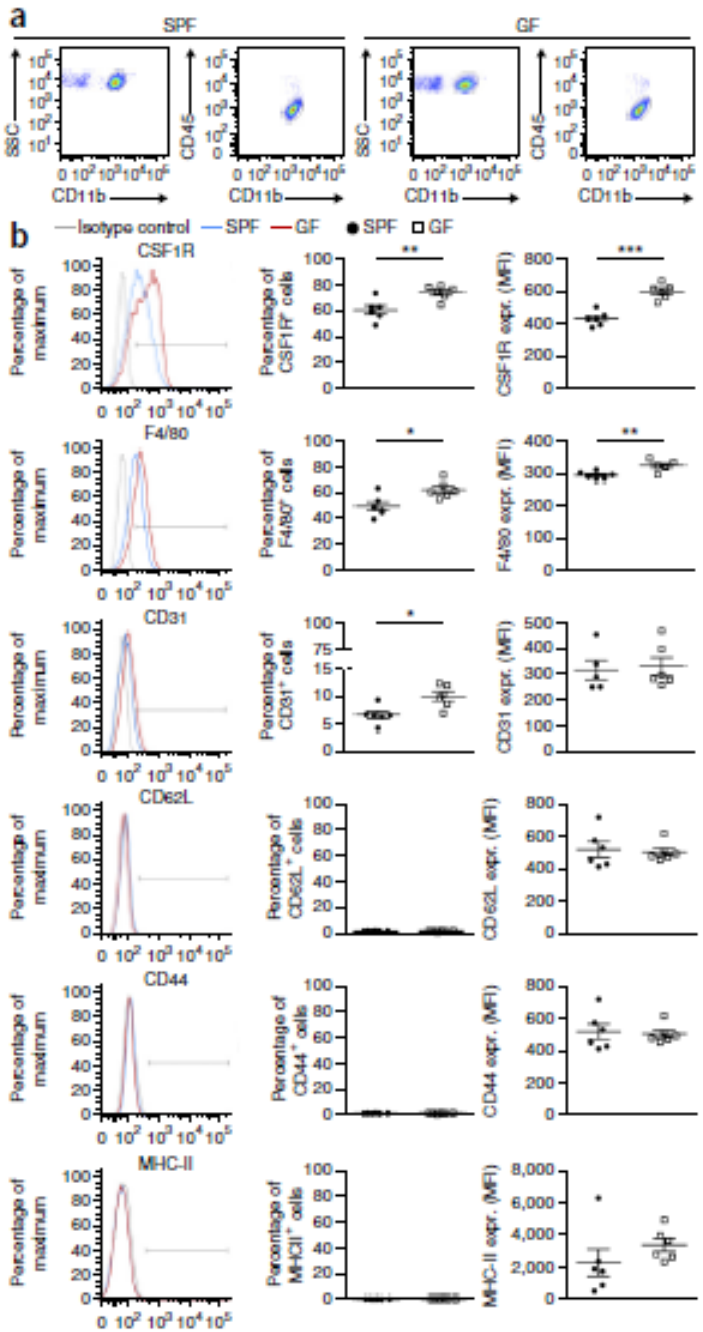


Figure 2 Increased expression of maturation and activation marker in GF microglia. (**a**) Gating strategy for flow cytometric analysis of CD11b$^+$ CD45^{lo} microglia from GF and SFP mice. Representative dot plots obtained from three independent experiments are shown. SSC, side scatter. (**b**) Representative cytometry graphs of the maturation and activation marker CSF1R, F4/80, CD31, CD44, CD62L and MHC class II on microglia from GF mice (red lines), SFP mice (blue lines) and isotype controls (gray lines). In addition, quantifications of the percentages (%) of positively labeled cells and MFIs are depicted. Each symbol represents data from one mouse, with six investigated mice per group. Data are presented as mean ± s.e.m. Significant differences were determined by an unpaired *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Data are representative of three independent experiments. *P* values: CSF1R (percentage of positive cells), 0.0068; CSF1R (MFI), <0.0001; F4/80 (percentage of positive cells), 0.0134; F4/80 (MFI), 0.0047; CD31 (percentage of positive cells), 0.0103.

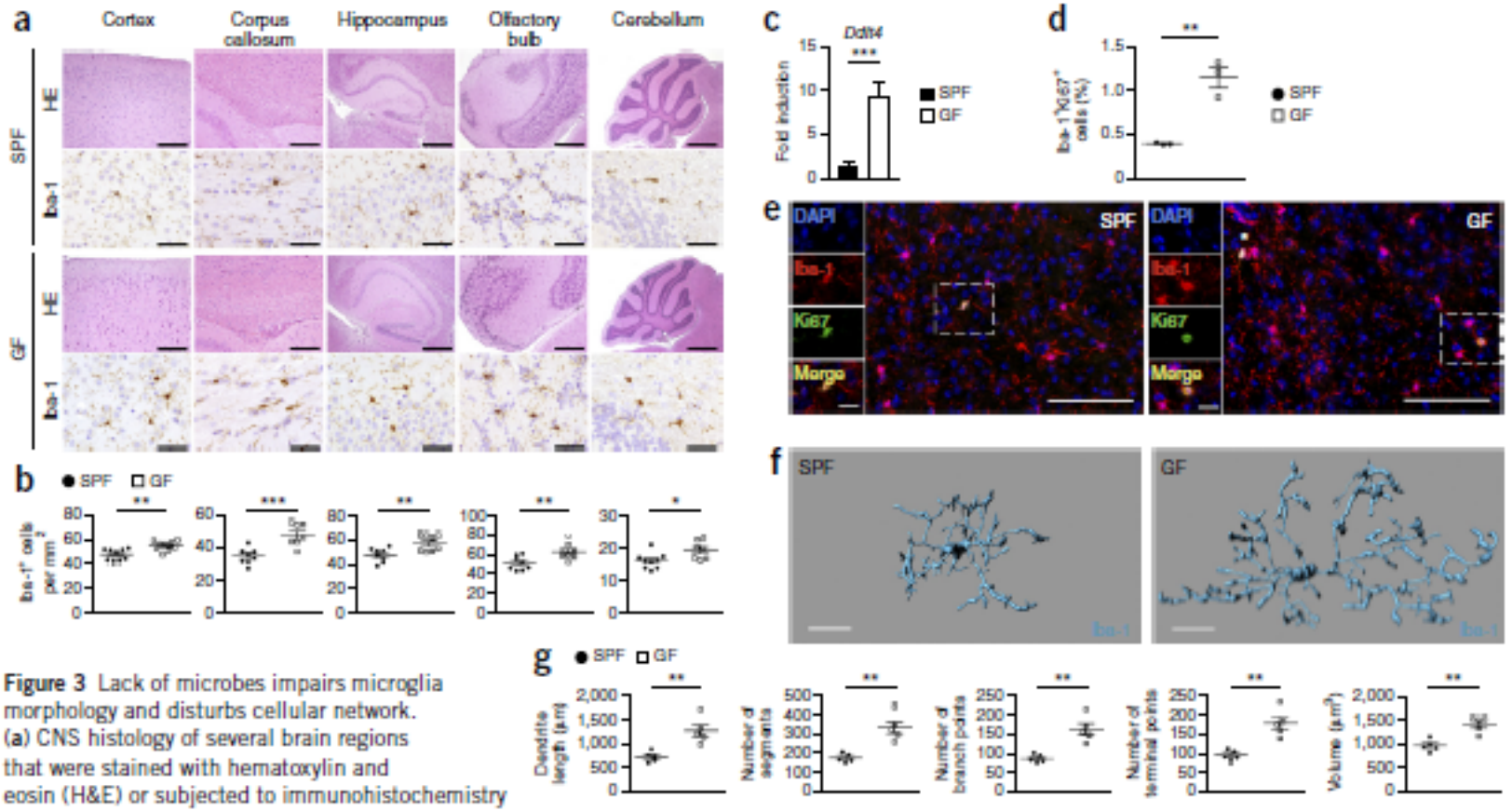


Figure 3 Lack of microbes impairs microglia morphology and disturbs cellular network. (**a**) CNS histology of several brain regions that were stained with hematoxylin and eosin (H&E) or subjected to immunohistochemistry for Iba-1 to detect microglia. Scale bars represent 200 µm (H&E, cortex and corpus callosum), 500 µm (H&E, hippocampus and olfactory bulb), 1 mm (H&E, cerebellum) and 50 µm (Iba-1). Representative pictures from nine mice per group are displayed. (**b**) Number of Iba-1$^+$ ramified parenchymal microglia in different localizations of the CNS. Each symbol represents data from one mouse, with nine mice per group. Three to four sections per mouse were examined. Data are presented as mean ± s.e.m. Data are representative of two independent experiments. Significant differences were determined by an unpaired *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). *P* values: cortex, 0.0024; corpus callosum, 0.0008; hippocampus, 0.0073; olfactory bulb, 0.0092; cerebellum, 0.0246. (**c**) Expression of *Ddit4* mRNA measured by qRT-PCR in microglia isolated from SPF (black bar) or GF (white bar) mice. Data are presented as mean ± s.e.m. with five samples in each group. Significant differences were determined by an unpaired *t* test (***$P = 0.0002$). Data are representative of two independent experiments. (**d**) Quantification of proliferating Iba-1$^+$ Ki67$^+$ double-positive parenchymal microglia was performed on cortical brain slices. Each symbol represents one mouse, with three mice per group. Three to four sections per mouse were examined. Data are presented as mean ± s.e.m. Significant differences were determined by an unpaired *t* test (**$P = 0.0033$). (**e**) Fluorescence microscopy of Iba-1$^+$ (red) microglia, the proliferation marker Ki67 (green) and DAPI (4′,6-diamidino-2-phenylindole, blue). Overview and magnification are shown. Scale bars represent 100 µm (overview) and 20 µm (inset). (**f**,**g**) Three-dimensional reconstruction (scale bars represent 15 µm, **f**) and Imaris-based automatic quantification of cell morphometry (**g**) of cortical Iba-1$^+$ microglia. Each symbol represents one mouse with at least three measured cells per mouse. Five mice per group were analyzed. Data are presented as mean ± s.e.m. Significant differences were determined by an unpaired *t* test (**$P < 0.01$). *P* values: dendrite length, 0.0035; number of segments, 0.0012; number of branch points, 0.0012; number of terminal points, 0.0012; volume, 0.0011.

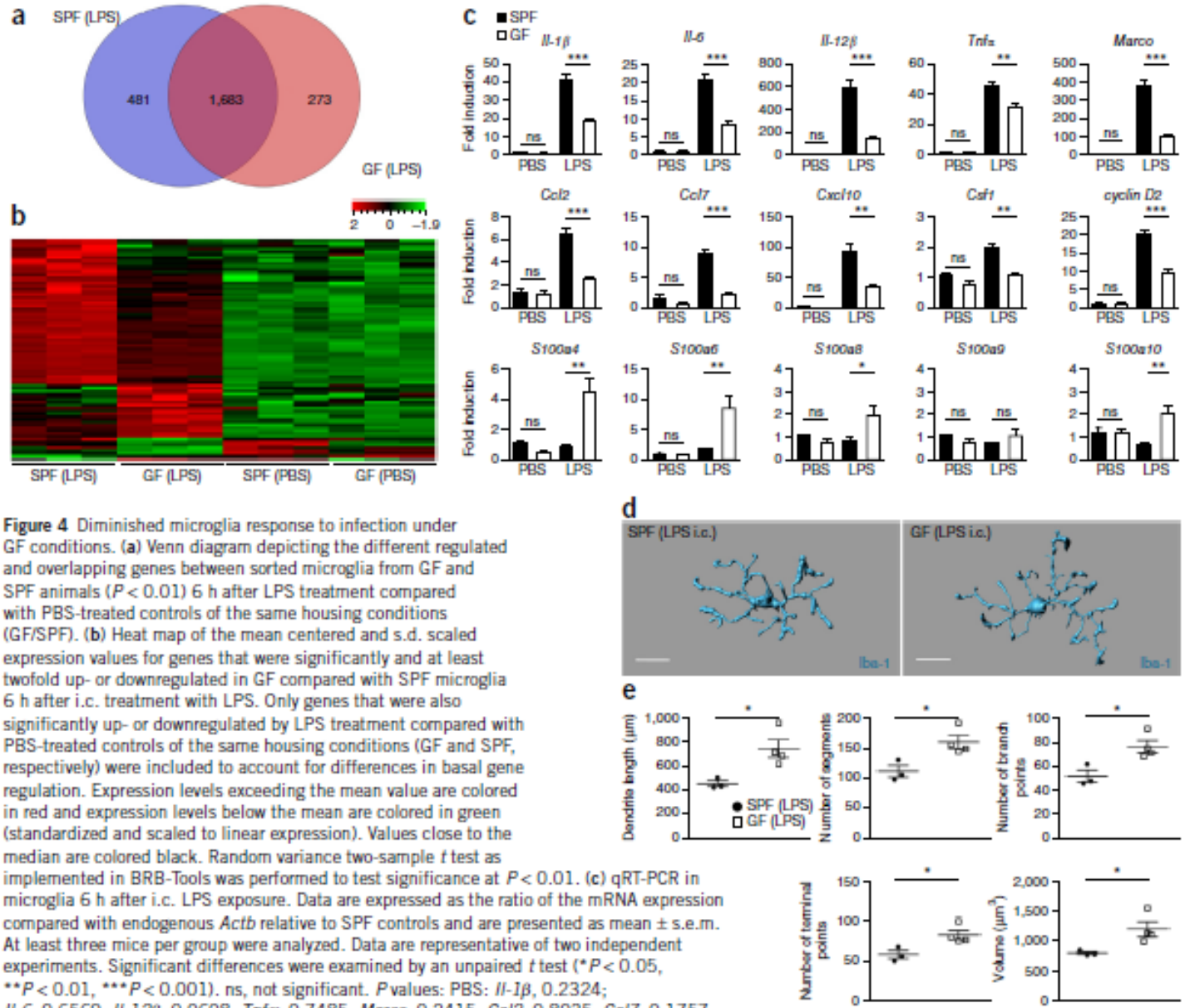


Figure 4 Diminished microglia response to infection under GF conditions. (**a**) Venn diagram depicting the different regulated and overlapping genes between sorted microglia from GF and SPF animals ($P < 0.01$) 6 h after LPS treatment compared with PBS-treated controls of the same housing conditions (GF/SPF). (**b**) Heat map of the mean centered and s.d. scaled expression values for genes that were significantly and at least twofold up- or downregulated in GF compared with SPF microglia 6 h after i.c. treatment with LPS. Only genes that were also significantly up- or downregulated by LPS treatment compared with PBS-treated controls of the same housing conditions (GF and SPF, respectively) were included to account for differences in basal gene regulation. Expression levels exceeding the mean value are colored in red and expression levels below the mean are colored in green (standardized and scaled to linear expression). Values close to the median are colored black. Random variance two-sample *t* test as implemented in BRB-Tools was performed to test significance at $P < 0.01$. (**c**) qRT-PCR in microglia 6 h after i.c. LPS exposure. Data are expressed as the ratio of the mRNA expression compared with endogenous *Actb* relative to SPF controls and are presented as mean ± s.e.m. At least three mice per group were analyzed. Data are representative of two independent experiments. Significant differences were examined by an unpaired *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). ns, not significant. *P* values: PBS: *Il-1β*, 0.2324; *Il-6*, 0.6569; *Il-12β*, 0.0608; *Tnfα*, 0.7485; *Marco*, 0.2415; *Ccl2*, 0.8035; *Ccl7*, 0.1757; *Cxcl10*, 0.2138; *Csf1*, 0.1224; *cyclin D2*, 0.8405; *S100a4*, 0.1279; *S100a6*, 0.1169; *S100a8*, 0;1169; *S100a9*, 0.2677; *S100a10*, 0.8502. LPS: *Il-1β*, 0.0001; *Il-6*, 0.0005; *Il-12β*, 0.0001; *Tnfα*, 0.0050; *Marco*, 0.0001; *Ccl2*, 0.0001; *Ccl7*, 0.0003; *Cxcl10*, 0.0055; *Csf1*, 0.0028; *cyclin D2*, 0.0003; *S100a4*, 0.0033; *S100a6*, 0.0050; *S100a8*, 0.0425; *S100a9*, 0.2136; *S100a10*, 0.0028. (**d**,**e**) Three-dimensional reconstruction (scale bar represents 15 µm, **d**) and Imaris-based automatic quantification of cell morphometry (**e**) of cortical Iba-1$^+$ microglia 6 h after i.c. treatment with LPS. Each symbol represents one mouse with at least three measured cells per mouse. Three SPF and four GF animals were investigated. Data are presented as mean ± s.e.m. Significant differences were determined by an unpaired *t* test (*$P < 0.05$). *P* values: dendrite length,