Brain, Behavior, and Immunity 122 (2024) 547–554

Contents lists available at ScienceDirect

Brain Behavior and Immunity

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

Short Communication

Multispecies probiotic intake during pregnancy modulates neurodevelopmental trajectories of offspring: Aiming towards precision microbial intervention

Tatiana Siegler Lathrop [a], Sarah Perego [b], Thomaz F.S. Bastiaanssen [c], Saskia van Hemert [d], Ioannis S. Chronakis [a], Rochellys Diaz Heijtz [b,*]

[a] Technical University of Denmark, DTU-Food, Research Group for Food Production Engineering, Laboratory of Nano-BioScience, Denmark
[b] Department of Neuroscience, Karolinska Institutet, Stockholm Sweden
[c] Department of Psychiatry, Amsterdam UMC, the Netherlands
[d] Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands

ARTICLE INFO

Keywords:
Maternal probiotic
Early-life
Anxiety-like behavior
Peptidoglycan transporter
Gut microbiota
IL-10

ABSTRACT

Recent research highlights the pivotal role of the maternal gut microbiome during pregnancy in shaping offspring neurodevelopment. In this study, we investigated the impact of maternal intake of a multispecies probiotic formulation during a critical prenatal window (from gestational day 6 until birth) on neurodevelopmental trajectories in mice. Our findings demonstrate significant and persistent benefits in emotional behavior, gut microbiota composition, and expression of tight junction-related genes, particularly in male offspring, who exhibited heightened sensitivity to the probiotic intervention compared to females. Additionally, we observed elevated gene expression levels of the anti-inflammatory cytokine *IL-10* and the oxytocin receptor (*Oxtr*) in the prefrontal cortex (PFC) of exposed juvenile offspring; however, these changes persisted only in the adult male offspring. Furthermore, the sustained increase in the expression of the proton-coupled oligopeptide transporter 1 (*PepT1*), which is involved in the transport of bacterial peptidoglycan motifs, in the PFC of exposed male offspring suggests a potential mechanistic pathway underlying the observed sex-dependent effects on behavior and gene expression. These results underscore the potential of prenatal multispecies probiotic interventions to promote long-term neurodevelopmental outcomes, with implications for precision microbial reconstitution aimed at promoting healthy neurodevelopment and behavior.

1. Introduction

Environmental factors during early life can profoundly influence the formation and function of neural circuits, a phenomenon known as developmental programming. One such influential factor is the gut microbiota, the trillions of microorganisms residing in the mammalian gastrointestinal tract, which have evolved mutualistic relationships with their hosts (Davenport et al., 2017). Preclinical studies using germ-free (GF) rodent models have highlighted the profound impact of gut microbiota on various neurodevelopmental processes, including blood–brain barrier (BBB) formation, microglial development and maturation, myelination, synaptogenesis, and complex behaviors such as social interactions and anxiety (Cryan et al., 2019). These investigations have revealed sex-dependent effects and identified critical developmental periods during which microbiota-brain interactions exert significant influence.

The maternal environment during pregnancy is widely recognized to play a crucial role in prenatal brain development and long-term neurodevelopmental trajectories, impacting susceptibility to mental health disorders from childhood to adulthood (Al-Haddad et al., 2019). Over the past decades, studies have shed light on the impact of disturbances in maternal gut microbiota during pregnancy, resulting from perinatal challenges such as stress, inflammation, dietary changes, and antibiotic exposure on the neurodevelopmental trajectories of offspring (Bolte et al., 2022). For instance, exposure to a high-fat diet during pregnancy induces behavioral and gut microbial alterations in offspring linked to decreased oxytocin production and synaptic plasticity (Sgritta et al., 2019). Recent studies have demonstrated that maternal gut microbiota

* Corresponding author.
E-mail address: rochellys.heijtz@ki.se (R. Diaz Heijtz).

https://doi.org/10.1016/j.bbi.2024.08.050
Received 23 April 2024; Received in revised form 15 August 2024; Accepted 24 August 2024
Available online 26 August 2024

derived metabolites such as 5-aminovalerate and hippurate can reach the fetal brain, influencing its programming (Vuong et al., 2020). Together, these observations suggest that prenatal life is not only a critical time-window of developmental vulnerability but also a time of opportunity in which therapeutic interventions may have a maximal effect on neural circuits and subsequent functions.

While a rapidly growing number of studies have shown beneficial effects of probiotic intervention during early life on stress-related hormones, intestinal barrier integrity, and maladaptive behaviors across different animal models (Codagnone et al., 2019), limited information exists about the impact of probiotic intake exclusively during pregnancy on the neurodevelopmental trajectories of offspring (Cuinat et al., 2022), but see (Surzenko et al., 2020). There is growing interest in using probiotics during pregnancy to foster healthy brain development in offspring at increased risk for neurodevelopmental disorders, such as autism spectrum disorder. However, the International Scientific Association for Probiotics and Prebiotics recently highlighted a lack of data on potential long-term adverse effects of early-life probiotic use (Merenstein et al., 2023).

In the current study, we aimed to investigate the impact of multispecies probiotic supplementation during a critical prenatal window of neurodevelopment (from gestational day 6 until birth) on anxiety-like behavior and gene expression (*e.g.*, genes involved in gut barrier and BBB integrity) in both male and female offspring during the juvenile and adulthood periods. Additionally, we examined the enduring effects on the gut microbiota composition of adult offspring. For this purpose, we selected Ecologic® Panda, a mixture of *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, and *Lactococcus lactis* W58. These strains can induce the production of the anti-inflammatory cytokine interleukin (IL)-10 *in vitro* (Niers et al., 2005). Moreover, Ecologic® Panda has been successfully used in a clinical trial involving pregnant women and postnatally in their infant offspring who were at high familial risk of developing allergic diseases (Niers et al., 2009). Interestingly, studies have found a significant association between allergic diseases and an increased risk of anxiety and depression in children and adolescents (Lu et al., 2012), underscoring the potential of prenatal microbiome-based strategies in promoting healthy neurodevelopment and behavior in offspring.

2. Material and methods

2.1. Animals

Time-mated pregnant C57BL6/rRj dams were obtained from Janvier-Labs and housed individually under standard conditions at the Comparative Medicine Biomedicum (KM-B), Karolinska Institutet. All procedures were approved by the Ethics Committee on Animal Research (Dnr. 12837-2020), Stockholm North, and complied with European Communities Council Directive 86/609/EEC.

2.2. Probiotic treatment

The probiotic formulation Ecologic® Panda contains four different probiotic strains (*B. bifidum* W23, *B. lactis* W51, *B. lactis* W52, and *L. lactis* W58) in a carrier matrix of rice starch and maltodextrin. Both the multispecies probiotic formulation and maltodextrin (used as a control) were provided by Winclove Probiotics B.V., Amsterdam, the Netherlands. Oral administration via drinking water was selected over oral gavage to minimize stress to the mice. Ten pregnant dams were randomly assigned to receive either Ecologic® Panda (n = 5; 8 × 10^7 CFU/ml) or a maltodextrin control (n = 5) in their drinking water from gestational day 6 until birth. All solutions were refreshed daily.

2.3. Experimental design

The date of birth was considered postnatal day (P) 0. Offspring were weaned at postnatal day (P) 20 and housed in same-sex, same-treatment groups. Behavioral testing occurred during the juvenile period (P22–P28) and early adulthood (P56–P62). We tested six mice per sex and developmental stage. Each mouse was randomly selected from one of five independent mothers (n = 5 per group for both the Ecologic® Panda and control groups), ensuring that each mother contributed at most two pups per group.

2.4. Behavioral testing

Testing occurred between 0900 and 1700 h. Equipment was sanitized before and after each mouse.

2.4.1. Open field (OF) test

Mice were placed in a 48 cm × 48 cm OF box (ActiMot detection system, TSE, Bad Homburg, Germany) for 15 min. Parameters recorded included distance traveled, number of rears, and center time.

2.4.2. Light/dark box (LDB) test

Mice explored a two-compartment box (24 cm × 24 cm each) with one side illuminated and the other dark for 5 min. Parameters measured included time, distance traveled, and rears in each compartment.

2.4.3. Elevated plus maze (EPM) test

The EPM apparatus (Kinder Scientific) comprised black Plexiglas with two open arms (36 × 5 cm), two enclosed arms (36 × 5 cm), and a central area (5 × 5 cm), elevated 64 cm above the floor. Mice freely explored all zones for 5 min, with time in each zone measured.

2.5. RNA Extraction and qRT-PCR

RNA was extracted from prefrontal cortex (PFC) and colon tissue samples using the QIAGEN RNeasy Mini Kit (Qiagen), with mechanical lysing via the bead method (0.5 mm zirconium beads, Precellys Evolution Touch homogenizer, Bertin Technologies). qRT-PCR assays were conducted on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad), and data analysis was performed using CFX Manager software (Bio-Rad), as described previously (Morel et al., 2023).

2.6. 16S rRNA gene sequencing and analysis

DNA was extracted from the cecal microbiota of adult mice using the QIAmp PowerFecal Pro DNA kit (Qiagen AB, Sweden), with mechanical lysing via the bead method (0.5 mm zirconium beads, Precellys Evolution Touch homogenizer, Bertin Technologies). The V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified, sequenced using the Illumina MiSeq 250 platform, and analyzed by Novogene (UK).

Further data handling was done in R (version 4.1.2) with the RStudio GUI (version 1.4.1717), as described previously (Lynch et al., 2023). Alpha diversity was analyzed with linear models. Principal component analysis (PCA) was applied to centered log-ratio (clr) transformed values for visualizing beta diversity. Beta diversity, computed using Aitchison distance (Euclidean distance of clr-transformed counts), was assessed with PERMANOVA from the vegan library with 1000 permutations. Differential abundance of taxa was examined through linear models. Plotting was done using ggplot2. R scripts are accessible at https://github.com/thomazbastiaanssen/Tjazi.

2.7. Statistical analysis

Data analysis utilized GraphPad Prism 10 software or R (version 4.1.2). Results are presented as means ± standard error of the mean. Significant outliers were identified using Grubb's test ($\alpha = 0.05$). Data from the OF, LDB, and EPM tests underwent mixed-effects analysis of variance (ANOVA). Post hoc comparisons employed the Bonferroni/Dunn test for significant ANOVA effects. Gene expression data were

analyzed using two-way ANOVA and subjected to multiple testing corrections with the Bonferroni method. For the microbiome data, the Benjamini-Hochberg procedure was used to correct for multiple testing with a false discovery rate (FDR) threshold of 0.1. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Prenatal probiotic exposure did not affect offspring body weight

No significant differences in body weights were observed between prenatally probiotic-exposed offspring and their respective control groups during both juvenile and early adulthood (Supplementary Fig. 1A and B).

3.2. Prenatal probiotic exposure reduces anxiety-like behavior in adult male offspring

To assess anxiety-like behaviors, we conducted the LDB and OF tests on both juvenile and adult offspring, with the EPM test exclusively used in adults. In the LDB test, no significant differences in compartment time were found in probiotic-exposed juvenile offspring ($P > 0.1$, Fig. 1A and C). However, adult male offspring spent significantly more time in the light compartment and less time in the dark compartment compared to controls (Treatment × Compartment interaction: [$F(1, 16) = 33.73$, $P < 0.0001$]; Bonferroni's post hoc comparison: control vs. probiotic: $P = 0.0017$ and $P = 0.0016$ for light and dark compartments, respectively (Fig. 1B)). No differences were found in adult females ($P > 0.1$, Fig. 1D).

In the OF test, prenatally exposed juvenile offspring spent significantly more time in the center zone for both males and females [Males: Treatment: $F(1, 29) = 10.58$, P=0.0029; Time: $F(2, 29) = 4.118$, $P = 0.0266$; Females: Treatment: $F(1, 29) = 5.601$, $P = 0.0248$, Time: $F(2, 29) = 4.089$, $P = 0.0273$]. Bonferroni's post hoc comparison revealed significant differences between control and probiotic groups at specific time intervals (Males: $P = 0.0392$ at 15 min interval; Females: $P = 0.0412$ at 10 min interval) (Fig. 1E). No significant differences were observed in adult offspring ($P > 0.1$, Fig. 1F).

In the EPM, the only significant difference found was that probiotic-exposed adult female offspring spent significantly more time in the closed zone (Treatment × Zones interaction: [$F(2, 30) = 5.727$, $P = 0.0078$]; Bonferroni's post hoc comparison: control vs. probiotic: $P = 0.0115$) (Fig. 1H).

3.3. Prenatal probiotic increases exploratory activity in adult female offspring

Locomotor and exploratory activity, assessed in the OF by total distance traveled and number of rears, respectively. The only statistically significant differences observed was in the number of rears in exposed adult females (Treatment: [$F(1, 29) = 5.601$, $P = 0.0248$]); Bonferroni's post hoc comparison: control vs. probiotic: $P = 0.0043$ at 10 min interval) (Supplementary Fig. 1H).

3.4. Prenatal probiotic exposure alters the expression of key genes in the PFC

To investigate the potential impact of perinatal multispecies probiotic exposure on BBB integrity, we analyzed the gene expression of several tight-junction-related genes, including claudin-1 (*Cldn1)*, claudin-3 *(Cldn3)*, claudin-5 (*Cldn5)*, junctional adhesion molecule A (*JAM-A)*, zonula occludens-1 (*ZO-1)*, and occludin (*Ocln)*. In prenatally probiotic-exposed juvenile males, we observed a significant increase in the expression of *Cldn5* and *JAM-A* ($P < 0.0001$ and $P = 0.0086$, respectively; Fig. 2A), both of which are crucial for maintaining BBB integrity. Conversely, prenatally exposed adult males showed a significant increase only in the expression of *Cldn*3 ($P = 0.0124$; Fig. 2B). Among exposed juvenile female offspring, there was a significant increase in the expression of *Cldn3* ($P = 0.0072$; Fig. 2C), while no changes were observed in the adult female offspring.

Prenatal exposure to multispecies probiotics did not affect the expression of brain-derived neurotrophic factor (*Bdnf*; $P > 0.1$; data not shown) or ionized calcium-binding adaptor molecule 1 (*Iba-1*; $P > 0.1$; Fig. 1A–D), which are markers of synaptic activity and microglial activation, respectively. We also assessed the expression of triggering receptor expressed on myeloid cells 2 (*Trem-2*), a microglial marker involved in immune modulation (Yeh et al., 2017). No significant differences were found in juvenile and adult male or juvenile female offspring ($P > 0.1$; Fig. 2A–C). However, *Trem-2* was significantly upregulated in adult female offspring ($P = 0.0034$; Fig. 2D). Additionally, *IL-10* expression, an anti-inflammatory cytokine known to be induced by the multispecies probiotic formulation (Niers et al., 2005), increased significantly in juvenile and adult male offspring ($P = 0.001$ and $P < 0.0001$; Fig. 2A and B). Elevated *IL-10* levels were also observed in exposed juvenile females ($P = 0.0215$; Fig. 2C), but not in adult females.

We also evaluated the expression of oxytocin receptor (*Oxtr*), which is implicated in anxiety-like behavior (Amico et al., 2008), and peptide transporter 1 (*PepT1*; also known as Slc15a1), which transports peptidoglycan (PGN) motifs (Gonzalez-Santana and Diaz Heijtz, 2020). *Oxtr* expression was significantly increased in juvenile females ($P = 0.0194$; Fig. 2C) and adult males ($P = 0.0028$; Fig. 2B). Similarly, *PepT1* expression increased significantly in exposed juvenile and adult male offspring ($P = 0.0023$ and $P < 0.0001$; Fig. 2A and B), as well as in juvenile female offspring ($P < 0.0001$; Fig. 2C), but not in adult females.

3.5. Prenatal probiotic exposure alters intestinal tight junction and mucosal-related gene expression

To investigate the impact of prenatal multispecies probiotic exposure on intestinal barrier integrity, we assessed the gene expression of tight junction and mucosal-related genes in juvenile and adult offspring (Fig. 2E–H). Prenatally exposed juvenile males exhibited significant increase in the expression of *Cldn1* and *Cldn5* ($P = 0.0015$ and $P = 0.0462$, respectively; Fig. 2E). In contrast, the expression of mucin 2 (*Muc2)* and mucin (*Muc3)* genes was upregulated in adult male offspring ($P < 0.0001$ and $P = 0.0032$, respectively; Fig. 2F). On the other hand, prenatally exposed juvenile females exhibited a significant decrease in *Cldn3* ($P = 0.0113$), whereas *Cldn5* was upregulated in adult females ($P = 0.0121$; Fig. 2H).

3.6. Prenatal probiotics produce enduring effects on adult gut microbiota composition

The overall proportion of the gut microbiota at the genus level for prenatally-exposed juvenile and adult offspring, as well as their respective controls, is shown in Fig. 3A. PCA identified significant differences in beta diversity, indicating variations in the overall diversity (Fig. 3B). However, there were no significant differences in various parameters of alpha diversity (Chao 1, Simpson's index, and Shannon entropy), which describe the community complexity in terms of species richness and evenness, when analyzed at the genus level. The lasting effects of prenatal probiotic exposure were also reflected in changes in the relative abundance of several genera (Fig. 3D).

4. Discussion

Recent studies have emphasized the pivotal role of the maternal gut microbiota during pregnancy in shaping fetal neural circuits and subsequent developmental functions (Vuong et al., 2020), sparking interest in leveraging it to support healthy neurodevelopment in offspring. In our study, we demonstrate that prenatal intake of a multispecies probiotic during a crucial developmental window (from gestational day 6 until

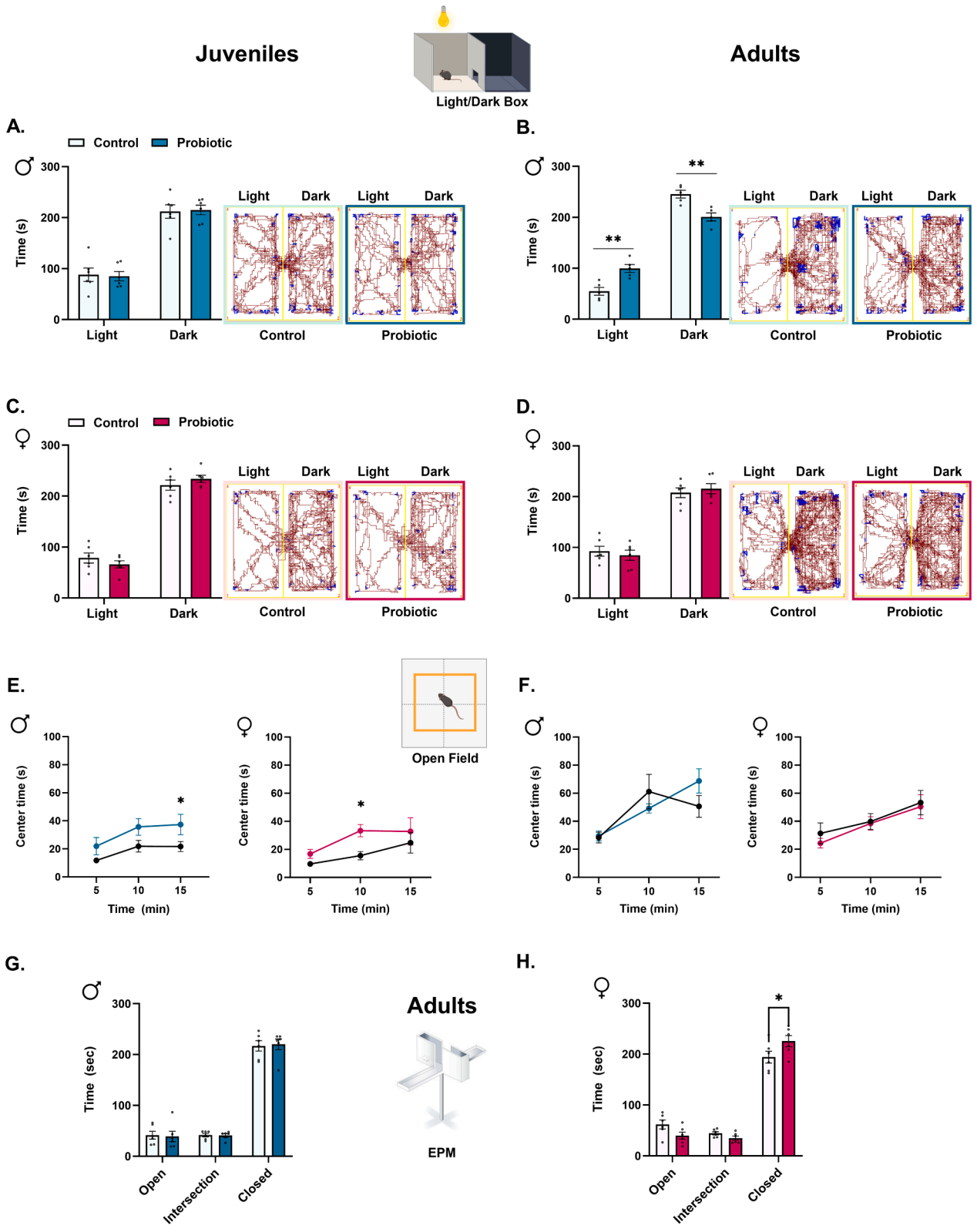

Fig. 1. Impact of maternal multispecies probiotic intake during pregnancy on offspring anxiety-like behavior. A–D. Bars represent the time (seconds) spent in the light and dark compartments, along with representative movement patterns during a 5-min Light/Dark Box (LDB) test by male and female juvenile (A and C) and adult (B and D) offspring. E –F. Average time (seconds) spent in the center of an Open Field (OF) box, measured in 5-min bins across a 15-min session by male and female juvenile (E) and adult (F) offspring. (G and H) Average time (seconds) spent in the different zones of the elevated plus maze (EPM) by prenatally probiotic-exposed adult male (G) and female (H) offspring. All data (A–H) are presented as means (±SEM; n = 5–6 per group). *$P < 0.05$; **$P < 0.001$ compared to their respective control group.

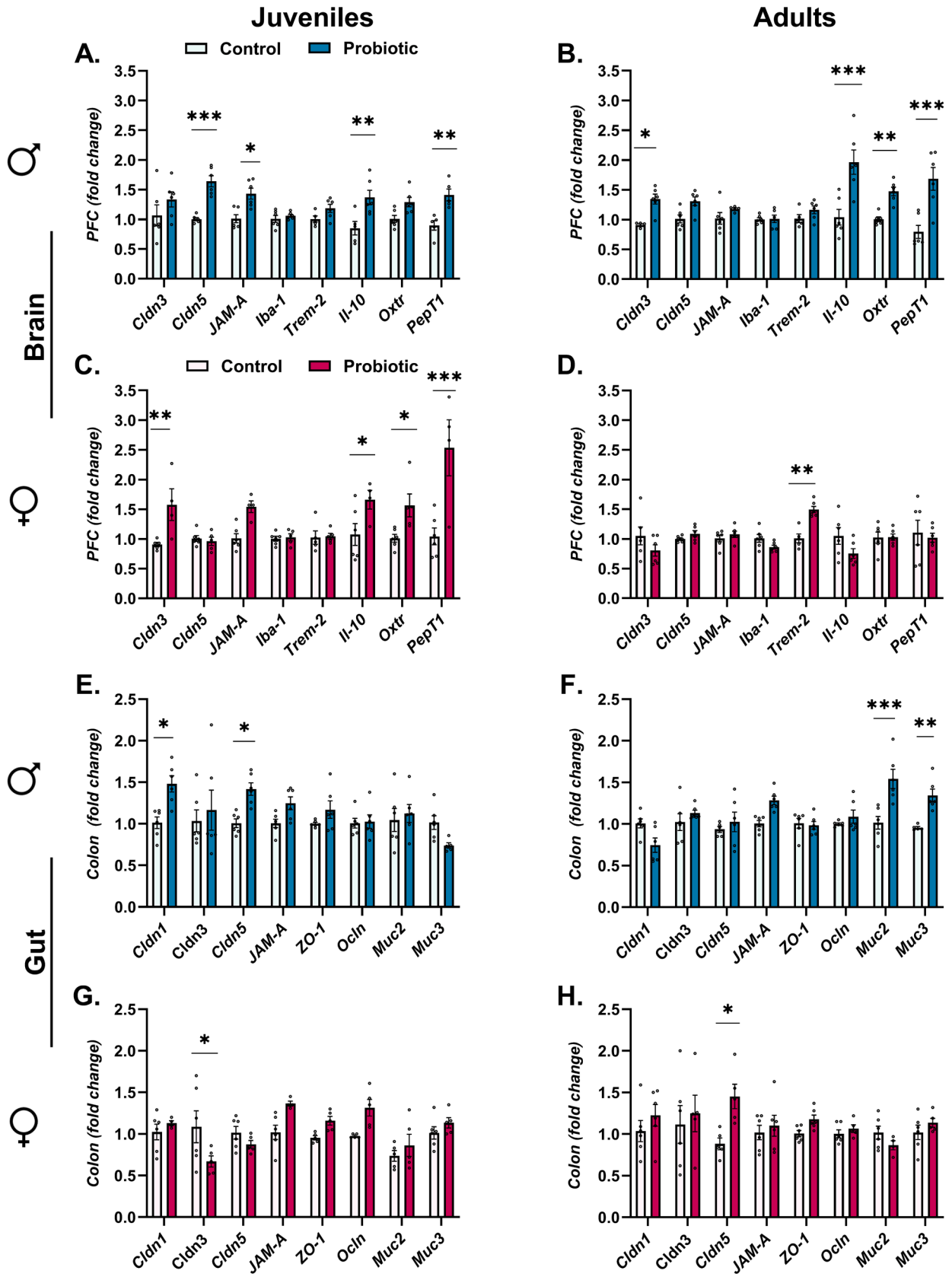

Fig. 2. Impact of maternal multispecies probiotic intake during pregnancy on the expression of various key genes in the prefrontal cortex (PFC) and colons of offspring. A–D. Expression of key genes in the PFC of juvenile and adult male (A and B) and female (C and D) offspring. E–H. Expression of tight junction and mucosal-related genes in the colons of juvenile and adult male (E and F) and female (G and H) offspring. All data (A–H) are presented as means (±SEM; n = 5–6 per group) with adjusted P-values after correction for multiple comparisons. *$P < 0.05$; **$P < 0.001$ compared to their respective control group.

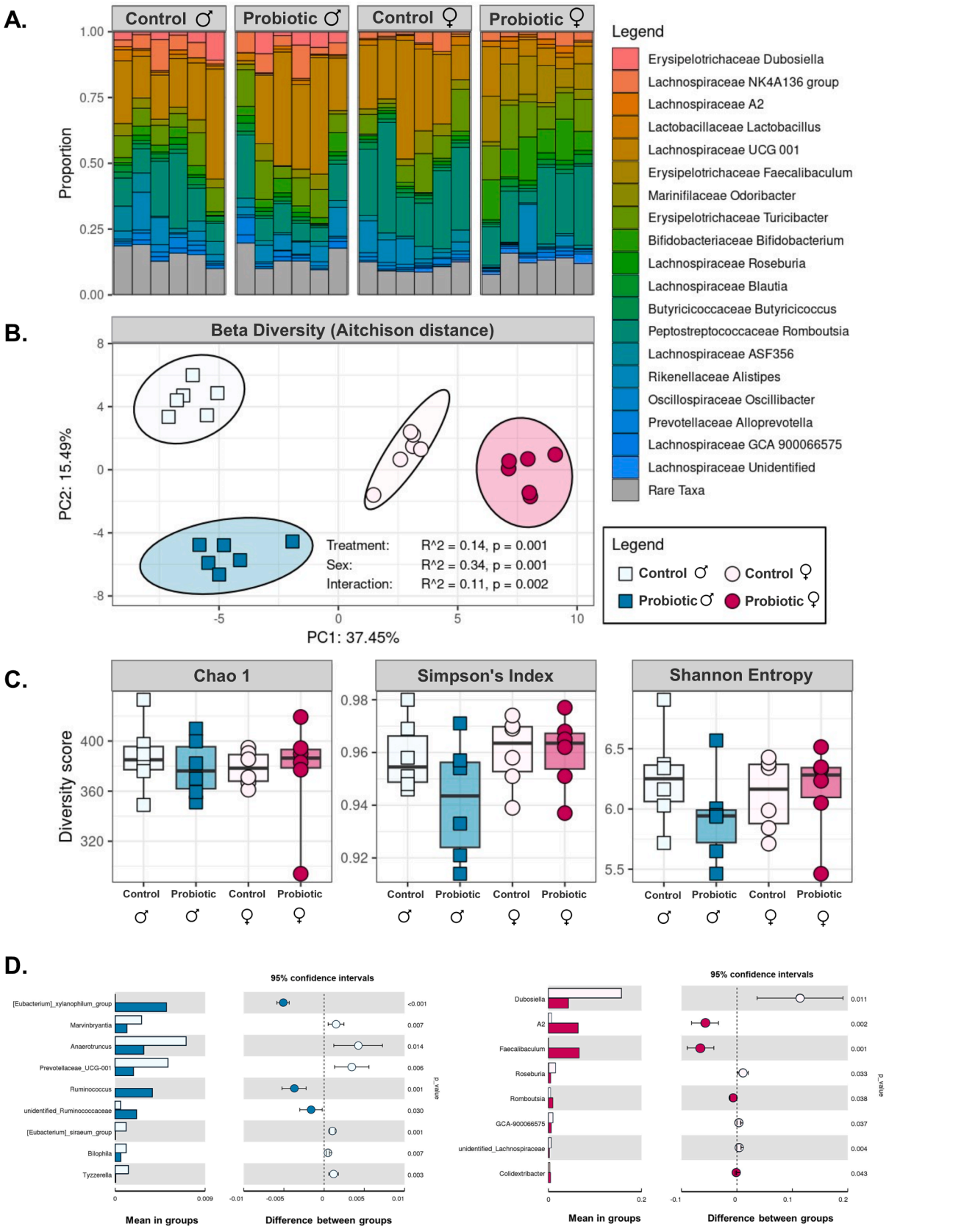

Fig. 3. Long-lasting changes in caecal microbiota composition of prenatally probiotic-exposed adult offspring. A. Relative proportion at the genus level. B. Principal component analysis (PCA) of beta-diversity. Prenatal probiotic exposure significantly altered beta-diversity in both male and female offspring. C. Mean (±SEM) alpha-diversity levels for adult mice, as measured by the Chao1, Simpson, and Shannon entropy indices. D. Relative abundance and 95 % confidence intervals of bacterial genera showing significant changes in prenatally probiotic-exposed adult male and female offspring compared to their respective controls.

birth) significantly alters the neurodevelopmental trajectories of offspring in a sex-dependent manner. Our results indicate distinct beneficial effects of prenatal multispecies probiotic intake on offspring emotional development, involving the regulation of genes related to intestinal and BBB integrity, as well as the anti-inflammatory cytokine *IL-10*. Notably, these changes were more prominent and lasting in prenatally probiotic-exposed male offspring than in the exposed female offspring. Consistent with the reduction in anxiety-like behavior observed in the LDB test, we noted an upregulation of *Oxtr* gene expression in the PFC of prenatally probiotic-exposed adult male offspring. These results underscore the significant impact of maternal probiotic administration during pregnancy, revealing notable neurodevelopmental shifts in key genes that may affect behavior.

Prenatal exposure to multispecies probiotics had enduring effects on the composition of the gut microbiota in adult offspring, which may contribute to long-term behavioral outcomes. We observed a significant increase in beneficial bacterial genera in exposed adult male offspring, including the *Eubacterium xylanophilum* and *Ruminococcus*, known for producing anti-inflammatory metabolites like short-chain fatty acids (Mukherjee et al., 2020; Reichardt et al., 2014). In adult female offspring, we noted a significant rise in *Lachnospiraceae bacterium A2* and *Faecalibaculum*, which are associated with reducing inflammation by promoting T regulatory cell development (van de Wouw et al., 2021). Conversely, *Dubosiella* genus, linked to intestinal inflammation from high-fat diet (Inaba et al., 2023), was significantly lower in exposed adult female offspring. These findings suggest that prenatal multispecies probiotic intervention may promote the growth of anti-inflammatory bacteria, leading to potential long-term benefits for the host.

Previous studies have highlighted the significant impact of gut microbiota on anxiety-like behavior in GF mice (Cryan et al., 2019). A prior study on *L. lactis*, a component of our multispecies probiotic formulation, reported reduced anxiety in prenatally exposed adult females (Surzenko et al., 2020). Our research revealed age- and sex-dependent effects of prenatal multispecies probiotic exposure on anxiety-like behavior, although these effects were not consistently observed across all tests. Specifically, juvenile males and females exhibited reduced anxiety-like behavior in the OF test. However, this effect was not uniform: in adulthood, only males showed a similar reduction in anxiety, as indicated by increased time spent in the light compartment of the LDB test. Conversely, adult females exhibited increased anxiety-like behavior, as evidenced by spending more time in the closed arms of the EPM. These discrepancies may reflect developmental changes, and/or hormonal influences, warranting further investigation.

Our study uncovered significant sex-dependent shifts in the expression of tight junction and mucosal-related genes in the colons of both juvenile and adult offspring, highlighting the crucial role of the maternal microbiome during pregnancy in shaping the intestinal barrier of offspring. While both male and female offspring exhibited increased expression of tight junction-related genes, changes in mucosal-related genes were observed only in the colons of exposed adult male offspring. Additionally, in the PFC, there were changes in the expression of tight junction-related genes in prenatally probiotic-exposed juvenile offspring, with some effects persisting into adulthood in the male offspring. However, future studies are needed to determine potential sex- and age-dependent changes in gut or BBB permeability following prenatal probiotic exposure.

Consistent with previous *in vitro* studies on the multispecies probiotic Ecologic® Panda (Niers et al., 2005), we observed upregulation of *IL-10* gene expression in the PFC of both juvenile male and female offspring, as well as in adult male offspring, from dams exposed to the probiotic. *IL-10* is known for its role in modulating the immune response by suppressing pro-inflammatory cytokines and inhibiting the activation of inflammatory cells. Accordingly, we did not detect changes in the expression of *Iba-1* and *Trem-2*, markers of microglial activation, in the PFC of juvenile and adult male offspring. However, *Trem-2* levels were elevated in adult female offspring, indicating a potential sex-specific inflammatory response to probiotic exposure, which warrants further investigation.

Oxytocin is a neuropeptide that plays a crucial role not only on social behaviors and stress regulation, but also anxiety-like behavior (Gottschalk and Domschke, 2018). Our findings suggest that *Oxtr* expression in the developing PFC of male offspring is more sensitive to specific signals from the probiotic formulation, as the increase in *Oxtr* persisted into adulthood in males but not females. Previous research indicates that a subset of *Oxtr*-responsive interneurons (OxtrINs) in the PFC modulates social and anxiety-like behaviors in a sex-dependent manner (Li et al., 2016). Future studies should explore whether OxtrINs are particularly susceptible to prenatal probiotic exposure, thus contributing to the behavioral changes observed in this study.

The mechanisms underlying the effects of probiotics during prenatal brain development are not fully understood. Recent studies indicate that bacterial cell components, such as PGN motifs, act as key molecular signals in gut microbiota-host crosstalk in early life (Gonzalez-Santana and Diaz Heijtz, 2020). Strikingly, we observed persistent upregulation of the PGN transporter, *PepT1*, in the PFC of prenatally probiotic-exposed juvenile and adult male offspring. Our probiotic formulation contains three *Bifidobacterium* spp. abundant in anhydro-PGN motifs, which exhibit potent anti-inflammatory activity *in vitro* (Kwan et al., 2024). These observations raise the possibility that PGN motifs derived from maternal probiotic intakes during pregnancy could contribute to neurodevelopmental programming in a sex-dependent manner.

In summary, our study demonstrates that prenatal exposure to multispecies probiotics has lasting positive effects on emotional behavior, gut microbiota composition, and gene expression, particularly with persistent changes observed in male offspring. These findings highlight the potential for precision microbial reconstitution as a strategy to support healthy neurodevelopment by modulating the maternal microbiome during pregnancy. Future research with larger sample sizes, detailed behavioral assessments, and advanced omics techniques is needed to identify modulated brain circuits and elucidate the underlying molecular mechanisms and signaling pathways.

CRediT authorship contribution statement

Tatiana Siegler Lathrop: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Sarah Perego:** Investigation. **Thomaz F.S. Bastiaanssen:** Validation, Visualization. **Saskia van Hemert:** Funding acquisition, Writing – review & editing, Methodology. **Ioannis S. Chronakis:** Writing – review & editing, Supervision, Funding acquisition, Project administration, Writing – original draft. **Rochellys Diaz Heijtz:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Funding from Olle Engkvist Foundation (226-0123), Swedish Medical Council (2018-06232), IFD (7076-00053B), DTU (Alliance-stipendier), and HHINT Kickstarter for PPP (LSHI 19004) supported this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.08.050.

References

Al-Haddad, B.J.S., Oler, E., Armistead, B., Elsayed, N.A., Weinberger, D.R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., Adams Waldorf, K.M., 2019. The fetal origins of mental illness. Am. J. Obstet. Gynecol. 221, 549–562.

Amico, J.A., Miedlar, J.A., Cai, H.M., Vollmer, R.R., 2008. Oxytocin knockout mice: a model for studying stress-related and ingestive behaviours. Prog. Brain Res. 170, 53–64.

Bolte, E.E., Moorshead, D., Aagaard, K.M., 2022. Maternal and early life exposures and their potential to influence development of the microbiome. Genome Med. 14, 4.

Codagnone, M.G., Stanton, C., O'Mahony, S.M., Dinan, T.G., Cryan, J.F., 2019. Microbiota and neurodevelopmental trajectories: role of maternal and early-life nutrition. Ann. Nutr. Metab. 74 (Suppl 2), 16–27.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K. E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Cruz-Pereira, J.S., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons, S.E., Hyland, N., Clarke, G., Dinan, T.G., 2019. The microbiota-gut-brain axis. Physiol. Rev. 99, 1877–2013.

Cuinat, C., Stinson, S.E., Ward, W.E., Comelli, E.M., 2022. Maternal intake of probiotics to program offspring health. Curr. Nutr. Rep. 11, 537–562.

Davenport, E.R., Sanders, J.G., Song, S.J., Amato, K.R., Clark, A.G., Knight, R., 2017. The human microbiome in evolution. BMC Biol. 15, 127.

Gonzalez-Santana, A., Diaz Heijtz, R., 2020. Bacterial peptidoglycans from microbiota in neurodevelopment and behavior. Trends Mol. Med. 26, 729–743.

Gottschalk, M.G., Domschke, K., 2018. Oxytocin and anxiety disorders. Curr. Top. Behav. Neurosci. 35, 467–498.

Inaba, T., Yamashiro, K., Kurita, N., Ueno, Y., Miyamoto, N., Hira, K., Nakajima, S., Kijima, C., Nakaguro, R., Urabe, T., Hattori, N., 2023. Microbial lipopolysaccharide-induced inflammation contributes to cognitive impairment and white matter lesion progression in diet-induced obese mice with chronic cerebral hypoperfusion. CNS Neurosci. Ther. 29 (Suppl 1), 200–212.

Kwan, J.M.C., Liang, Y., Ng, E.W.L., Sviriaeva, E., Li, C., Zhao, Y., Zhang, X.L., Liu, X.W., Wong, S.H., Qiao, Y., 2024. In silico MS/MS prediction for peptidoglycan profiling uncovers novel anti-inflammatory peptidoglycan fragments of the gut microbiota. Chem. Sci. 15, 1846–1859.

Li, K., Nakajima, M., Ibanez-Tallon, I., Heintz, N., 2016. A Cortical Circuit for Sexually Dimorphic Oxytocin-Dependent Anxiety Behaviors. Cell 167 (60–72), e11.

Lu, Y., Mak, K.K., van Bever, H.P., Ng, T.P., Mak, A., Ho, R.C., 2012. Prevalence of anxiety and depressive symptoms in adolescents with asthma: a meta-analysis and meta-regression. Pediatr. Allergy Immunol. 23, 707–715.

Lynch, C.M.K., Cowan, C.S.M., Bastiaanssen, T.F.S., Moloney, G.M., Theune, N., van de Wouw, M., Florensa Zanuy, E., Ventura-Silva, A.P., Codagnone, M.G., Villalobos-Manriquez, F., Segalla, M., Koc, F., Stanton, C., Ross, P., Dinan, T.G., Clarke, G., Cryan, J.F., 2023. Critical windows of early-life microbiota disruption on behaviour, neuroimmune function, and neurodevelopment. Brain Behav. Immun. 108, 309–327.

Merenstein, D., Pot, B., Leyer, G., Ouwehand, A.C., Preidis, G.A., Elkins, C.A., Hill, C., Lewis, Z.T., Shane, A.L., Zmora, N., Petrova, M.I., Collado, M.C., Morelli, L., Montoya, G.A., Szajewska, H., Tancredi, D.J., Sanders, M.E., 2023. Emerging issues in probiotic safety: 2023 perspectives. Gut Microbes 15, 2185034.

Morel, C., Martinez Sanchez, I., Cherifi, Y., Chartrel, N., Diaz Heijtz, R., 2023. Perturbation of maternal gut microbiota in mice during a critical perinatal window influences early neurobehavioral outcomes in offspring. Neuropharmacology 229, 109479.

Mukherjee, A., Lordan, C., Ross, R.P., Cotter, P.D., 2020. Gut microbes from the phylogenetically diverse genus Eubacterium and their various contributions to gut health. Gut Microbes 12, 1802866.

Niers, L., Martin, R., Rijkers, G., Sengers, F., Timmerman, H., van Uden, N., Smidt, H., Kimpen, J., Hoekstra, M., 2009. The effects of selected probiotic strains on the development of eczema (the PandA study). Allergy 64, 1349–1358.

Niers, L.E., Timmerman, H.M., Rijkers, G.T., van Bleek, G.M., van Uden, N.O., Knol, E.F., Kapsenberg, M.L., Kimpen, J.L., Hoekstra, M.O., 2005. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. Clin. Exp. Allergy 35, 1481–1489.

Reichardt, N., Duncan, S.H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K.P., Flint, H.J., Louis, P., 2014. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. ISME J. 8, 1323–1335.

Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., Costa-Mattioli, M., 2019. Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. Neuron 101 (246–259), e246.

Surzenko, N., Pjetri, E., Munson, C.A., Friday, W.B., Hauser, J., Mitchell, E.S., 2020. Prenatal exposure to the probiotic Lactococcus lactis decreases anxiety-like behavior and modulates cortical cytoarchitecture in a sex specific manner. PLoS One 15, e0223395.

van de Wouw, M., Walsh, C.J., Vigano, G.M.D., Lyte, J.M., Boehme, M., Gual-Grau, A., Crispie, F., Walsh, A.M., Clarke, G., Dinan, T.G., Cotter, P.D., Cryan, J.F., 2021. Kefir ameliorates specific microbiota-gut-brain axis impairments in a mouse model relevant to autism spectrum disorder. Brain Behav. Immun. 97, 119–134.

Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., Hsiao, E.Y., 2020. The maternal microbiome modulates fetal neurodevelopment in mice. Nature 586, 281–286.

Yeh, F.L., Hansen, D.V., Sheng, M., 2017. TREM2, Microglia, and Neurodegenerative Diseases. Trends Mol. Med. 23, 512–533.