Modifying the maternal microbiota alters the gut–brain metabolome and prevents emotional dysfunction in the adult offspring of obese dams

Daniel E. Radford-Smith, Fay Probert, Philip W. J. Burnet, and Daniel C. Anthony
1Department of Pharmacology, University of Oxford, Oxford OX1 3QZ, United Kingdom; 2Department of Chemistry, University of Oxford, Oxford OX1 3TA, United Kingdom; 3Department of Psychiatry, Warneford Hospital, University of Oxford, Oxford OX3 7JX, United Kingdom; and 4Laboratory of Psychiatric Neurobiology, I.M. Sechenov First Moscow State Medical University, Moscow 119435, Russia

Edited by John Cryan, Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland; received May 7, 2021; accepted January 18, 2022

Maternal obesity disturbs brain–gut–microbiota interactions and induces negative affect in the offspring, but its impact on gut and brain metabolism in the offspring (F1) are unknown. Here, we tested whether perinatal intake of a multispecies probiotic could mitigate the abnormal emotional behavior in the juvenile and adult offspring of obese dams. Untargeted NMR-based metabolomic profiling and gene-expression analysis throughout the gut–brain axis were then used to investigate the biology underpinning behavioral changes in the dams and their offspring. Prolonged high-fat diet feeding reduced maternal gut short-chain fatty acid abundance, increased markers of peripheral inflammation, and decreased the abundance of neuroactive metabolites in maternal milk during nursing. Both juvenile (postnatal day [PND] 21) and adult (PND112) offspring of obese dams exhibited increased anxiety-like behavior, which were prevented by perinatal probiotic exposure. Maternal probiotic treatment increased gut butyrate and brain lactate in the juvenile and adult offspring and increased the expression of prefrontal cortex PFKFB3, a marker of glycolytic metabolism in astrocytes. PFKFB3 expression correlated with the increase in gut butyrate in the juvenile and adult offspring. Maternal obesity reduced synaptophysin expression in the adult offspring, while perinatal probiotic exposure increased expression of brain-derived neurotrophic factor. Finally, we showed that the resilience of juvenile and adult offspring to anxiety-like behavior was most prominently associated with increased brain lactate abundance, independent of maternal group. Taken together, we show that maternal probiotic supplementation exerts a long-lasting effect on offspring neuroplasticity and the offspring gut–liver–brain metabolome, increasing resilience to emotional dysfunction induced by maternal obesity.

Significance

Maternal obesity is a growing public health concern and is linked to an increased risk of neurodevelopmental and psychiatric disorders in humans. Despite accumulating evidence for the important role maternal microbes play during gestation and nursing, the longitudinal effect of maternal nutrition on offspring metabolism across the gut–brain axis at different ages remains unexplored. We provide evidence for the protective efficacy of perinatal probiotic exposure against increased anxiety-like behaviors induced by maternal obesity. Protection was maintained into adulthood, which may be mediated by the enhancement of brain energy metabolites and an increase in gut butyrate. These findings reveal the importance and long-lasting role of maternally derived microbiota and metabolites in increasing resilience to mood disorders in the offspring.

Depression is a common and debilitating disorder affecting all ages and demographics in a manner that is largely independent of economic factors (1, 2). Similarly, maternal obesity is a growing public health concern in Europe (3) and in the United States (4), and, in low- and middle-income countries, its prevalence is rapidly increasing (5–7). Though accumulating evidence points to a biological linkage between obesity and depression (8), few thorough investigations have been undertaken into how maternal obesity affects offspring behavior (9).

In humans, affective behavioral problems in childhood, such as depression and anxiety, are more likely to occur after gestational and early life exposure to maternal obesity (10, 11). To better understand the relationship between maternal diet-induced obesity and the behavior of offspring, animal models are used to allow greater control over nutrition and eliminate confounding genetic, environmental, and socioeconomic factors. Such preclinical studies have progressed our understanding of the underlying mechanisms linking maternal high-fat diet (HFD) intake and offspring neurodevelopment (12). For example, the important research of Sullivan et al. (13) has linked changes in maternal HFD consumption during gestation with decreased cerebrospinal fluid levels of 5-hydroxytryptamine and increased anxiety-like behavior in juvenile, female nonhuman primate (NHP) offspring, but abnormalities were not assessed in adulthood. Neuroplastic changes may be linked to early exposure to hepatic inflammation as a consequence of maternal HFD-induced obesity (14).

The last decade has seen the microbiota–gut–brain axis emerge as a pivotal regulator of neurodevelopment (15–17) and obesity (18). Maternal diet and obesity affect the composition of the maternal and neonatal gut microbiome in humans (19–21) and rodents (22). Moreover, maternal gut dysbiosis induced by diet (23) or antimicrobial treatment (24) has been shown to affect the offspring microbiome, as well as the brain and behavior of the offspring. Despite these discoveries, no studies have investigated whether maternal probiotic intake may counter the adverse effects of maternal obesity via changes to the microbiota–gut–brain axis. Lipid-lowering drugs remain strongly contraindicated during pregnancy (25), whereas probiotics are safe to administer (26).

Author contributions: D.E.R.-S., P.W.J.B., and D.C.A. designed research; D.E.R.-S. and F.P. performed research; D.E.R.-S. and F.P. analyzed data; and D.E.R.-S., F.P., P.W.J.B., and D.C.A. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission. J.C. is a guest editor invited by the Editorial Board. This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1To whom correspondence may be addressed. Email: daniel.anthony@pharm.ox.ac.uk or philburnet@psych.ox.ac.uk.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2108581119/-/DCSupplemental.

PNAS 2022 Vol. 119 No. 9 e2108581119

Published February 23, 2022
In unchallenged, wild-type mice, maternal perinatal probiotic intake reduces offspring anxiety-like behaviors, alters brain N-methyl-D-aspartate receptor (NMDAR) gene expression, and increases fecal short-chain fatty acid (SCFA) levels (27). In the offspring of obese dams, microbial reconstitution with *Lactobacillus reuteri* can normalize brain dopaminergic activity and social behavior (23), although no insight into downstream changes in microbial, or brain, metabolism was presented. Subsequent rodent (24) and human (28) studies have identified neuromodulatory roles for specific microbial metabolites, as opposed to microbial species, whose metabolic potentials converge considerably (29). Furthermore, preliminary research also indicates a role for early life gut microbiota in the regulation of central astrocyte–neuron energy metabolism (30). Lactate, in particular, which is produced in the brain by astrocytes, makes an important contribution to neuronal energy homeostasis (31–33) and brain signaling (33, 34). Astrocyte dysfunction is a feature of clinical depression and generates depressive-like phenotypes in rodents (35), although it remains untested whether interventions targeting the early life gut–brain axis can restore or enhance brain energy metabolism, alter markers of neuronal plasticity, or increase emotional resilience.

Here, we confirm that maternal obesity induces negative affect in their offspring and show that these effects are long-lasting. Markers of immune activation were quantified in the liver of dams and offspring, and the relative expression of inflammatory and plasticity-related genes were evaluated in the prefrontal cortex (PFC). The protracted structural and functional plasticity of the PFC throughout life (36) renders it a prime target to investigate the adverse consequences of maternal obesity on offspring brain and behavior at juvenile and adult ages. Using untargeted NMR-based metabolomic profiling, we also sought to discover how maternal obesity disturbs brain–gut metabolic interactions in male and female offspring during development and maturity and whether these effects could be mitigated by perinatal exposure to a multispecies probiotic. Our findings reveal a long-lasting protective effect of maternal probiotic intake on offspring anxiety-like behavior, which we show exhibits a persistent association with offspring brain lactate levels.

**Results**

**Chronic HFD Exposure Induces Maternal Obesity and Perturbs the Microbiota–Gut–Brain-Axis Metabolome.** Mix-effects modeling of time, HFD, and probiotic treatment grouping between 4 and 12 wk of age showed that pre gravid mice fed an HFD gained significantly more weight over time than mice fed a control diet (CD) (Fig. 1), independent of probiotic treatment, which led to an obese phenotype (>20% heavier than CD) at the time of mating (SI Appendix, Fig. S1). Litter size was reduced in obese dams, relative to lean dams (SI Appendix, Fig. S2). Two-way ANOVA revealed that maternal obesity induction (CD) and offspring sex (male vs. female) were significant main effects of time and both main effects were significant in post hoc comparisons. Lean dams relative to CD dams (SI Appendix, Figs. S10–S12) had reduced brain lactate levels compared to male and female offspring of CD dams. In the liver, male offspring had significantly lower lactate levels compared to females (Fig. S13, SI Appendix).

**Maternal Obesity Alters Maternal Behaviors and Markers of Innate Immunity Postpartum.** During the first week of nursing, maternal HFD-induced obesity led to a significant increase in time spent away from the nest and reduced the frequency of nest building (SI Appendix, Fig. S10) relative to dams fed the CD, independent of probiotic treatment and with no interaction. In the forced swim test (FST), a significant diet × probiotic treatment interaction was identified, whereby HFD/vehicle dams exhibited a significantly reduced latency to immobility compared to CD/vehicle and CD/probiotic dams, whereas HFD/probiotic dams displayed a reduced latency to immobility only compared to CD/vehicle dams (SI Appendix, Fig. S11). No behavioral changes were seen in the light–dark box (LDB) or open field test (OFT) (SI Appendix, Fig. S11).

Two-way ANOVA revealed that the diet × probiotic treatment interaction was identified, whereby HFD/vehicle dams exhibited a significantly reduced latency to immobility compared to CD/vehicle and CD/probiotic dams, whereas HFD/probiotic dams displayed a reduced latency to immobility only compared to CD/vehicle dams (SI Appendix, Fig. S11). No behavioral changes were seen in the light–dark box (LDB) or open field test (OFT) (SI Appendix, Fig. S11)

Two-way ANOVA revealed that the diet × probiotic treatment interaction was identified, whereby HFD/vehicle dams exhibited a significantly reduced latency to immobility compared to CD/vehicle and CD/probiotic dams, whereas HFD/probiotic dams displayed a reduced latency to immobility only compared to CD/vehicle dams (SI Appendix, Fig. S11). No behavioral changes were seen in the light–dark box (LDB) or open field test (OFT) (SI Appendix, Fig. S11).
F0. Maternal probiotic treatment or vehicle control

**(A)** High fat diet (80% kcal) or control diet (10% kcal) feeding

| F0 | 6 weeks pre-feeding | 6 | Mating + Conception | Maternal Care | Observation |
|---|---|---|---|---|---|
| F1 | PND21 | 1200hrs | Maternal obesity increased passive stress coping independent of maternal probiotic treatment, with no interaction (Fig. 3A and B). Exposure to both perinatal HFD and probiotic increased offspring body weights in early life (**SI Appendix**, Fig. S14A), including at weaning age (**SI Appendix**, Fig. S14B), and, thus, there was no relationship between juvenile body weight and immobility in the FST, nor between OFT activity and the FST.

In the adult offspring, two-way ANOVA did not reveal an interaction for total time immobile (Fig. 3C), but there were independent main effects for both maternal diet and probiotic treatment, whereby maternal HFD increased time immobile in adult offspring relative to CD adult offspring independent of probiotic, and maternal probiotic treatment reduced time immobile relative to offspring exposed to vehicle, independent of maternal diet. Regarding latency to immobility, a significant interaction did occur. Tukey post hoc tests indicated that HFD/vehicle offspring exhibited a significantly reduced latency to immobility compared to adult offspring from each other group (Fig. 3D).

No weight differences persisted in adult male or female offspring (**SI Appendix**, Fig. S14C–E), and no relationship was identified between FST immobility and body weight.

Social interaction test. Behavior in the social interaction test (SIT) was assessed, in place of the LDB, in the adult offspring. Two-way ANOVA revealed that maternal obesity reduced social interaction at 8 wk of age, independent of maternal probiotic treatment and with no significant interaction between maternal diet and probiotic treatment (**SI Appendix**, Fig. S15).

**FST.** In the juvenile offspring, maternal obesity increased passive stress coping independent of maternal probiotic treatment, with no interaction (Fig. 3A and B). Exposure to both perinatal HFD and probiotic increased offspring body weights in early life (**SI Appendix**, Fig. S14A), including at weaning age (**SI Appendix**, Fig. S14B), and, thus, there was no relationship between juvenile body weight and immobility in the FST, nor between OFT activity and the FST.

In the adult offspring, two-way ANOVA did not reveal an interaction for total time immobile (Fig. 3C), but there were independent main effects for both maternal diet and probiotic treatment, whereby maternal HFD increased time immobile in adult offspring relative to CD adult offspring independent of probiotic, and maternal probiotic treatment reduced time immobile relative to offspring exposed to vehicle, independent of maternal diet. Regarding latency to immobility, a significant interaction did occur. Tukey post hoc tests indicated that HFD/vehicle offspring exhibited a significantly reduced latency to immobility compared to adult offspring from each other group (Fig. 3D).

No weight differences persisted in adult male or female offspring (**SI Appendix**, Fig. S14C–E), and no relationship was identified between FST immobility and body weight.

Social interaction test. Behavior in the social interaction test (SIT) was assessed, in place of the LDB, in the adult offspring. Two-way ANOVA revealed that maternal obesity reduced social interaction at 8 wk of age, independent of maternal probiotic treatment and with no significant interaction between maternal diet and probiotic treatment (**SI Appendix**, Fig. S15).
Maternal Perinatal Probiotic Treatment Increases the Delivery of Lactate and SCFAs to Offspring during Nursing. The composition of maternal milk during nursing was found to reflect the varying abundance of plasma and gut (microbial) metabolites of dams (Figs. 1F and 4A and B and SI Appendix, Figs. S16 and S17 and Tables S8 and S9). While two-way ANOVA of the key milk metabolites did not identify any significant interactions between diet and probiotic treatment (SI Appendix, Table S9), main effects of both diet and probiotic were identified. Specifically, we observed that milk from probiotic-fed dams contained a significantly elevated relative abundance of lactate, alanine, and SCFAs compared to milk from dams fed the vehicle control, a significantly elevated relative abundance of lactate, alanine, and SCFAs compared to milk from dams fed the vehicle control, and a significantly elevated relative abundance of lactate, alanine, and SCFAs compared to milk from dams fed the vehicle control. Post hoc Tukey tests revealed that HFD/vehicle offspring spent less time in center than CD/vehicle and HFD/probiotic adult offspring. (D) Rearing behavior in adult offspring was unchanged. Two-way ANOVA; data are presented as boxplots showing median, interquartile range, and min/max points. *P < 0.05 (indicating main effect of maternal probiotic treatment); **P < 0.01; ***P < 0.001 (indicating significant Tukey post hoc comparisons, computed only in the case of a significant diet × probiotic treatment interaction. Int., interaction; ns, not significant. n = 26 to 29 for juvenile offspring (13 to 15 male, 13 or 14 female) and 20 or 21 for adult offspring (10 or 11 male, 9 or 10 female) per group.

Perinatal Probiotic Exposure Increases Brain Lactate and Gut Butyrate in the Juvenile and Adult Offspring. Juvenile and adult offspring brain, liver, plasma, and fecal samples were interrogated for metabolite composition by using 1H NMR. No sex differences were observed in the F1 juvenile offspring (SI Appendix, Fig. S18A and B), and group differences in metabolites were unrelated to sex differences in the F1 adults (SI Appendix, Fig. S18C and D). PCA of all metabolites (relative abundance, z-scaled by sex) identified unsupervised group differences, in particular, as a result of maternal probiotic intake, in juvenile (Fig. 5A and PCA loadings plot, SI Appendix, Fig. S19) and adult (Fig. 5C and PCA loadings plot, SI Appendix, Fig. S20) offspring. PLS-DA was employed to identify the most important discriminatory metabolites. These are depicted in the PLS-DA loading plots (SI Appendix, Figs. S21–S28), complementary VIP scores in SI Appendix, Tables S10–S17, and summary atlases (SI Appendix, Fig. S16). From this analysis, metabolite heatmaps were generated for the juvenile (Fig. 5B) and adult (Fig. 5D) offspring, with corresponding analysis by two-way ANOVA in SI Appendix, Tables S18–S25.

Lactate levels, which were increased in the milk of probiotic-fed dams at postnatal day (PND) 4 (Fig. 4C), were also significantly elevated in the brain (see Fig. 8A), liver (SI Appendix, Table S19), and plasma (SI Appendix, Table S9B) of the juvenile (PND21) offspring relative to the offspring of vehicle-fed dams. This was independent of maternal diet and without a significant interaction. In the plasma, the increased lactate levels were within range of those previously reported during high-intensity treadmill exercise in mice (37–39). As in their mothers, the juvenile offspring exposed to the probiotic also exhibited elevated propionate and butyrate in the gut relative to vehicle offspring (see main effect, Fig. 8C and
Modifying the maternal microbiota alters the gut–brain metabolome and prevents emotional dysfunction in the adult offspring of obese dams

SI Appendix, Table S21 and Fig. S24). Other key brain metabolites altered by maternal diet and/or probiotic treatment are summarized in SI Appendix, Fig. S16 and Table S18. Briefly, there were significant interactions for brain creatine and brain acetate levels, where both HFD/vehicle and HFD/probiotic offspring had reduced levels compared to CD/vehicle offspring (Fig. 5C, D). However, only HFD/vehicle offspring had reduced levels relative to CD/probiotic offspring. Acetate levels were increased in the brain of HFD/probiotic offspring relative to all other groups, while both the HFD/vehicle and CD/probiotic offspring had reduced levels compared to the other groups.

In the adult female offspring (PND112), two-way ANOVA revealed that in the absence of a significant interaction, brain lactate remained elevated in the offspring exposed to maternal probiotic intake relative to vehicle controls. In the male adult offspring, there was a significant maternal diet × probiotic interaction, although no post hoc differences reached significance (see Fig. 8B). In the plasma (SI Appendix, Fig. S9C), lactate levels were not altered by maternal diet or probiotic treatment. There was, however, a main effect of maternal probiotic intake on gut butyrate (see Fig. S5D) and propionate levels, which remained increased in the adult offspring of probiotic-treated dams relative to vehicle offspring (Fig. S5D and SI Appendix, Table S25). Interestingly, tissue-specific PLS-DA suggested that maternal probiotic intake exerted the most lasting influence on the adult brain metabolome (SI Appendix, Fig. S25), as opposed to the liver, gut, or plasma metabolites (SI Appendix, Figs. S26–S28). Two-way ANOVA with Benjamini and Hochberg FDR correction indicated a main effect of maternal probiotic treatment relative to vehicle. In the juvenile offspring, two-way ANOVA (with the Bonferroni correction) revealed a main effect of maternal probiotic intake on GLUN2A expression, where expression was reduced in the offspring of probiotic-fed dams relative to the offspring of vehicle controls, independent of maternal diet with no interaction (Fig. 6A). In the same way,
GLUN2B expression was also reduced, but only in the female offspring (Fig. 6B). A significant maternal diet × probiotic interaction occurred for GLUN2C expression in the female juvenile offspring, whereby expression was increased only in HFD/vehicle female offspring compared to the juvenile female offspring from all other groups (Fig. 6C). GLUN1 and PSD-95, constituent postsynaptic NMDAR components, were unaltered in the juvenile offspring (SI Appendix, Table S26), and there were no significant effects or interactions between maternal diet and probiotic treatment on GLUN1/2A/2B/2C or PSD-95 gene expression in the F1 adults (SI Appendix, Table S27).

Expression of serotonin receptor subunits. There were no interactions or main effects of maternal obesity and probiotic on young or adult offspring serotoninergic receptor expression (SI Appendix, Tables S26 and S27).

Plasticity gene expression. Two-way ANOVA revealed a main effect of maternal probiotic intake on expression of the presynaptic density marker SYP, which was reduced in the male juvenile offspring of probiotic-fed dams relative to vehicle offspring, independent of maternal diet with no interaction (Fig. 6D). In the same way, CREB1 expression was increased in the male offspring (SI Appendix, Table S26). A significant interaction occurred for ZIF-268 expression in the male juvenile offspring. Post hoc Tukey tests showed that HFD/probiotic offspring had increased expression relative to both CD/probiotic and CD/vehicle offspring, while HFD/vehicle offspring had increased expression only relative to CD/probiotic offspring (Fig. 6E). With the absence of an interaction in the female offspring, there was a main effect of maternal diet, whereby expression was increased in the offspring of HFD-fed dams relative to CD (Fig. 6E). Other plasticity-related genes were unchanged at juvenile age (SI Appendix, Table S26).

In the adult offspring, there was a main effect of maternal probiotic intake on BDNF (Fig. 7A) and Δ FOSB (Fig. 7E) expression, where expression was increased in the offspring of probiotic-fed dams relative to vehicle, independent of maternal diet with no significant interaction or sex differences. SYP was decreased in the adult offspring of obese dams relative to lean dams as a main effect, independent of maternal probiotic treatment with no sex differences or interaction (Fig. 7B). Both ZIF-268 (Fig. 7C) and cFOS (Fig. 7D) expression were increased in the adult male offspring of probiotic-fed dams relative to the adult male offspring of vehicle-fed dams as a main effect without interaction. GSK3B and CREB1 expression were unaltered in the adult offspring (SI Appendix, Table S27).

Astrocytic metabolic activity. Two-way ANOVA revealed a main effect of maternal probiotic intake on PFKFB3 expression. PFKFB3 was increased in both the juvenile (Fig. 6F) and adult (Fig. 7F) offspring of dams treated with the probiotic compared to the offspring of vehicle controls, independent of maternal obesity and with no interaction or sex differences. ATP1A2 expression was not altered in the juvenile or adult offspring (SI Appendix, Tables S26 and S27).

Fig. 5. Offspring gut–brain metabolome reflects maternal metabotype influenced by diet and perinatal probiotic supplementation. (A) PCA scores plot of all juvenile offspring gut, liver, plasma, and brain metabolites at PND21. $R^2 = 0.51$ with six principal components. (B) Heatmap of discriminant metabolites contributing to the F1 juvenile (PND21) tissue-specific PLS-DA models. (C) PCA scores plot of all adult offspring gut, liver, plasma, and brain metabolites at PND112. $R^2 = 0.55$ with six principal components. (D) Heatmap of metabolites in the F1 adult (PND112) offspring, based on those metabolites altered in juvenile offspring. Top VIP metabolites that were significantly altered in at least one tissue were investigated in all tissues, n = 26 to 29 for offspring (13 to 15 male, 13 or 14 female) and 20 or 21 for adult offspring (10 or 11 male, 9 or 10 female) per group. Descriptive statistics are reported in SI Appendix, Figs. S19–S28 and Tables S10–S25.
**Proinflammatory cytokine expression.** Two-way ANOVA revealed that maternal probiotic supplementation, as a main effect, increased juvenile IL-6 in the PFC relative to the juvenile offspring of vehicle controls, independent of maternal obesity with no interaction or sex differences (*SI Appendix, Table S28*). Expression of TNF, TLR4, and IL-1β expression was unchanged, and there were no persistent changes in PFC cytokine expression in the adult offspring (*SI Appendix, Table S29*).

In contrast to PFC expression, a significant maternal diet × probiotic interaction was identified for liver IL-6 in the female juvenile offspring, with no significant interaction or main effects in male offspring. Post hoc tests revealed that expression was elevated in the offspring of HFD/vehicle dams relative to the offspring of all other conditions (*SI Appendix, Table S28*). There was also a main effect of maternal probiotic supplementation on reducing TLR4 expression in the liver of the juvenile offspring, relative to juvenile offspring from dams fed the vehicle, independent of maternal diet and with no sex differences and no significant maternal diet × probiotic interaction. In the adult male offspring, maternal obesity increased liver IL-1β and IL-6 expression relative to the adult male offspring of lean dams (main effect, *F*₁,₂₀ = 23.96). Two-way ANOVA; data are presented as boxplots showing median, interquartile range, and min/max points. Int., interaction.

Brain Lactate and Gut SCFAs Correlate with PFC Gene Expression. We further investigated how brain (*SI Appendix, Table S30*) and gut (*SI Appendix, Table S31*) metabolite levels (top three or four ranked by VIP score from the corresponding PLS-DA) correlated with altered PFC gene expression. In the juvenile offspring, there was a significant negative correlation between brain lactate and gut SCFAs.
Maternal probiotic treatment has lasting effects on astrocytic metabolism and neuronal plasticity. (A) Maternal probiotic treatment increased BDNF expression in the adult offspring of probiotic-fed dams relative to the adult offspring of dams given the vehicle [main effect, $F_{(1,44)} = 70.53$], independent of maternal diet. (B) Maternal HFD reduced SYP expression relative to maternal CD [main effect, $F_{(1,44)} = 12.86$]. The expression of ZIF-268 [main effect, $F_{(1,20)} = 34.99$] and cFos [main effect, $F_{(1,20)} = 25.17$] were significantly elevated in the male adult offspring of probiotic-treated dams, relative to vehicle offspring via a main effect of maternal probiotic treatment. $\Delta$FosB and PFKFB3 expression were both increased by maternal probiotic treatment, relative to the adult offspring of dams given the vehicle [main effects, $\Delta$FosB $F_{(1,42)} = 33.60$; PFKFB3 $F_{(1,42)} = 29.96$].

No significant maternal diet × probiotic treatment interactions reached significance. Data are presented as boxplots showing median, interquartile range, and min/max points. Int., interaction; ns, not significant. $^*P < 0.001$ (indicating main effect of maternal diet); $^\#P < 0.001$ (indicating main effect of maternal probiotic treatment). For all comparisons, note: Bonferroni-corrected significance level ($\alpha$) = 0.001. $n$ = 6 per sex per group. Where sex differences occurred, separate two-way ANOVAs were performed. Descriptive statistics are reported in SI Appendix, Tables S27 and S29.

Levels and expression of GLUN2A ($r = -0.47$, $q = 0.0080$), GLUN2B ($r = -0.50$, $q = 0.0080$), and SYP ($r = -0.40$, $q = 0.021$). A positive correlation existed between lactate and PFKFB3 ($r = 0.40$, $q = 0.021$; Fig. 8E). Increased alanine was associated with increased expression of IL-6 in the PFC ($r = 0.44$, $q = 0.013$). Creatine, the top brain metabolite reduced in the juvenile offspring of obese dams, was not significantly correlated with gene expression.

An increase in fecal SCFAs acetate ($r = 0.38$, $q = 0.025$), propionate ($r = 0.53$, $q = 0.0013$), and butyrate ($r = 0.44$, $q = 0.0088$; Fig. 8G) all significantly correlated with increased expression of PFKFB3 (SI Appendix, Table S31). Propionate negatively correlated with GLUN2A ($r = -0.35$, $q = 0.029$), GLUN2B ($r = -0.43$, $q = 0.0088$), and SYP ($r = -0.31$, $q = 0.047$) expression, while butyrate correlated negatively with GLUN2C ($r = -0.34$, $q = 0.038$) and SYP ($r = -0.37$, $q = 0.025$) expression.

For the same metabolite correlation analyses where PFC gene expression was also altered between groups in adult offspring, only fecal butyrate remained significantly correlated with PFC PFKFB3 ($r = 0.40$, $q = 0.027$; Fig. 8H and SI Appendix, Table S32). Lactate was not significant after correcting for the FDR ($r = 0.36$, $q = 0.057$; Fig. 8F and SI Appendix, Table S33).

Elevated Brain Lactate Accompanies the Therapeutic Effect of Early Life Probiotic Exposure on Anxiety-Like Behavior. Finally, we investigated how brain and gut metabolites—in particular, those that were discriminatory between offspring of lean, obese, and probiotic-supplemented dams—correlated with patterns of anxiety-like behavior. In both juvenile (area under the curve [AUC] 0.82) and adult (AUC 0.74) offspring, lactate was the most discriminatory brain metabolite between high (first quartile)
**Neuroscience**

**Fig. 8.** Key gut and brain metabolites altered by maternal probiotic treatment show correlations with a genetic marker of astrocytic metabolism and anxiety behavior. (A) In the juvenile offspring, two-way ANOVA revealed that maternal probiotic treatment increased brain lactate levels in both male [main effect, $F_{(1,53)} = 44.57$, $P < 0.05$] and female [main effect, $F_{(1,48)} = 60.07$, $P < 0.05$] relative to vehicle. (B) In the adult male offspring, there was a significant maternal diet × probiotic treatment interaction [$F_{(1,38)} = 5.32$, $P < 0.05$] for brain lactate. Note that post hoc Tukey analysis did not identify significant group differences ($P > 0.05$). In the adult female offspring, maternal probiotic intake increased brain lactate independent of diet [$F_{(1,34)} = 6.97$, $P < 0.05$]. Maternal probiotic treatment increased gut butyrate levels in both the juvenile offspring [main effect, $F_{(1,101)} = 13.83$, $P < 0.05$] (C) and the adult offspring [$F_{(1,72)} = 8.71$, $P < 0.05$] (D) independent of maternal diet and sex. A significant correlation (Spearman’s rank correlation coefficient) between brain lactate levels and PFC $PFKFB3$ expression was found for juvenile ($r = 0.40$, $n = 48$ [24F, 24M]) (E), but not for adult ($r = 0.36$, $n = 48$ [24F, 24M]) (F), offspring. Correlations for gut butyrate and PFC $PFKFB3$ expression were found for both juvenile ($r = 0.44$, $n = 48$ [24M, 24F]) (G) and adult ($r = 0.40$, $n = 48$ [24M, 24F]) (H) offspring. Receiver operating characteristic curves (I, juvenile; J, adult) for brain lactate concentration compared to other discriminatory brain metabolites as classifiers for high and low anxiety-like behavior in the OFT in juvenile ($n = 28$ low anxiety [10M, 18F], 28 high anxiety [15M, 13F]) and adult ($n = 20$ low anxiety [12M, 8F], high anxiety [5M, 15F]) offspring, respectively. Significant correlations between brain lactate levels and OFT time in center were found for juvenile ($r = 0.41$, $n = 111$ [57M, 54F]) (K) and adult ($r = 0.33$, $n = 80$ [42M, 38F]) (L) offspring. Offspring operating characteristic curves (I, juvenile; J, adult) for brain lactate concentration compared to other discriminatory brain metabolites as classifiers for high and low anxiety-like behavior in the OFT in juvenile ($n = 28$ low anxiety [10M, 18F], 28 high anxiety [15M, 13F]) and adult ($n = 20$ low anxiety [12M, 8F], high anxiety [5M, 15F]) offspring, respectively. Significant correlations between brain lactate levels and OFT time in center were found for juvenile ($r = 0.41$, $n = 111$ [57M, 54F]) (K) and adult ($r = 0.33$, $n = 80$ [42M, 38F]) (L) offspring. Receiver operating characteristic curves (I, juvenile; J, adult) for brain lactate concentration compared to other discriminatory brain metabolites as classifiers for high and low anxiety-like behavior in the OFT in juvenile ($n = 28$ low anxiety [10M, 18F], 28 high anxiety [15M, 13F]) and adult ($n = 20$ low anxiety [12M, 8F], high anxiety [5M, 15F]) offspring, respectively. Significant correlations between brain lactate levels and OFT time in center were found for juvenile ($r = 0.41$, $n = 111$ [57M, 54F]) (K) and adult ($r = 0.33$, $n = 80$ [42M, 38F]) (L) offspring. Receiver operating characteristic curves (I, juvenile; J, adult) for brain lactate concentration compared to other discriminatory brain metabolites as classifiers for high and low anxiety-like behavior in the OFT in juvenile ($n = 28$ low anxiety [10M, 18F], 28 high anxiety [15M, 13F]) and adult ($n = 20$ low anxiety [12M, 8F], high anxiety [5M, 15F]) offspring, respectively.

**Discussion**

The current study demonstrates that maternal obesity alters brain metabolism in the juvenile and adult offspring, with long-term effects on behavior and PFC plasticity gene expression. Maternal, perinatal multispecies probiotic supplementation ameliorated offspring anxiety-like behavior associated with maternal obesity in both the young (PND21) and adult (PND112) offspring. Additionally, early life probiotic exposure increased gut butyrate and brain lactate, reduced $GLUN2A/2B$ messenger RNA (mRNA), and elevated $PFKFB3$ expression [a marker of increased astrocytic metabolism through glycolysis (33)] in the PFC of young offspring relative to vehicle controls. $PFKFB3$ was also elevated in adult offspring exposed to perinatal probiotic supplementation relative to vehicle controls and remained correlated with

and low (fourth quartile) anxiety-like behavior, independent of experimental groupings (Fig. 8 I and J, respectively). Brain lactate levels correlated with reduced anxiety-like behavior in the open field across all juvenile ($r = 0.41$, $P < 0.0001$) and adult ($r = 0.33$, $P = 0.0034$) offspring (Fig. 8 K and L, respectively).

**Radford-Smith et al.**

Modifying the maternal microbiota alters the gut–brain metabolome and prevents emotional dysfunction in the adult offspring of obese dams

https://doi.org/10.1073/pnas.2108581119
gut butyrate levels. *BDNF* and other plasticity-related genes were increased in adulthood after early life probiotic exposure, whereas *SYN* levels were reduced in the adult offspring of obese dams relative to the offspring of lean dams. Together, these data show that the intake of a multispecies probiotic supplement during gestation and nursing has a lasting influence on behavior, neuroplasticity, and neurometabolism and can mitigate some of the behavioral symptoms of maternal obesity in the offspring.

The anxiolytic and antidepressant effects of perinatal probiotic exposure in this study are concordant with reports describing similar behavioral responses in rodent offspring: *Lactobacillus helveticus* NS8 intake in normal-weight dams from embryonic day (E) 13 to E22 during gestation has been shown to reduce offspring anxiety (40), and maternal perinatal probiotic intake (which encourages the growth of endogenous *Bifidobacterium spp.*.) reduced passive stress coping in the FST in adulthood (27). Reduced social behavior observed in the adult offspring of obese dams is associated with reduced *Bifidobacterium spp.* and *Lactobacillus spp.* (23), but reconstitution with microbiota of control offspring through coprophagia reversed these deficits. These findings, in combination with our results, emphasize the important role of maternal microbiota and metabolites on early life neurodevelopment and behavior (24).

In this study, expression of the *GLUN2A* and *GLUN2B* NMDAR subunits was reduced in the PFC of juvenile probiotic offspring. We previously found that maternal prebiotic supplementation reduced these subunits in the juvenile offspring hippocampus (27), and others have shown that prebiotic supplementation reduced GLUN2B protein in the frontal cortex of adult mice. Consistent with our present data in juvenile mice, these reported changes in gene expression were associated with reduced anxiety-like behavior (41). Thus, despite prebiotics and probiotics having distinct actions on the gut flora, the central mechanisms underlying their psychotropic effect may be similar. NMDAR antagonists, including those selective to the GLUN2B subunit, have antidepressant activity in mice (42) and humans (43, 44). While alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA) expression was not assessed in this study, a decrease in NMDAR expression may increase AMPA (non-NMDA glutamatergic) throughput relative to NMDAR in the PFC, which is one of the mechanisms thought to underly the persistent antidepressant effect of NMDAR antagonism (42). This is supported by the idea that antidepressant NMDAR antagonists affect presynaptic, rather than postsynaptic, circuits (45, 46), with antagonism ultimately enhancing PFC synaptic efficiency and glutamate release (46).

We show that expression of postsynaptic density marker PSD-95 was stable, while *SYN* expression, a presynaptic density marker, decreased in the male juvenile offspring of probiotic-fed dams alongside reductions in *GLUN2A* and *GLUN2B* expression relative to vehicle controls. These observations, together with the overall increase in cortical glutamate shown here in probiotic offspring, are largely consistent with the downstream effects of NMDAR antagonism in vivo (47).

Besides the increase in cortical glutamate, NMR-based metabolomic profiling revealed a substantial increase in L-lactate in the brain, liver, and plasma of juvenile offspring after maternal probiotic intake compared to vehicle controls, with no overt sex differences. Analysis of maternal milk at PND4 indicated increased lactate ingestion in probiotic compared to vehicle offspring during the first week of life, which may stem from increased levels of *Lactobacillus spp.* and *Bifidobacterium spp.* in the colon of dams receiving the probiotic (48). A direct link between maternal microbiota and increased brain lactate has not been reported, the importance of maternal microbiota and microbial metabolites in neurodevelopment during the perinatal period is recognized (24). L-lactate has antidepressant-like properties when administered to adult mice (49, 50), and the selective serotonin reuptake inhibitor fluoxetine has been shown to increase lactate release from astrocytes in vitro (51). Functioning independently as both a signaling molecule (33) and neuronal energy source (52, 53), the pleiotropic properties of lactate may have contributed to the early life changes in NMDAR gene expression (54) and behavior (49, 50). Here, the abundance of brain lactate, but not other brain metabolites, correlated highly with *GLUN2A/2B* and *PFKFB3* expression changes across all young male and female offspring and correlated with reduced anxiety-like behavior in both young and adult offspring. Lactate has been shown to directly modulate histone deacetylase (HDAC) activity in the mouse hippocampus, which was associated with reduced *GLUN2A* mRNA expression and resilience to chronic social defeat stress (49). *PFKFB3* is a master regulator of lactate production through the glycolytic pathway in astrocytes (55) and may be regulated in part by the microbiome (30). Here, we provide evidence supporting the role of microbes and microbial metabolites, in particular, brain lactate and gut butyrate, in regulating mood behavior and the expression of plasticity-related genes.

In juveniles, maternal obesity reduced brain and liver creatine levels, which were normalized after perinatal probiotic intake in the liver, but not the brain. A systematic review (56) collated data from studies employing in vivo brain proton magnetic resonance spectroscopy in major depressive disorder (MDD) patients and found that creatine and glutamate were significantly downregulated in MDD. This is consistent with the brain metabolite changes we found in the juvenile offspring of obese dams. Low dietary creatine intake is a clinical risk factor for depression (57), and creatine supplementation has shown promise as an adjunct supplement for MDD (58). A promising theory for the pathophysiology of synaptic dysfunction in psychiatric disorders is that plastic changes are constrained by brain energy deficits (59). This has been supported by preclinical (60) and clinical evidence (61) implicating reduced creatine in depression and reduced creatine kinase flux in bipolar disorder, respectively. The results from our metabolomic study support accumulating evidence connecting maternal obesity during pregnancy to long-term changes in central metabolism and behavior (12). We suggest that the increase in cortical lactate, the source of which may be astrocytic (via increased PFC lactate) (55), microbial (via increased milk lactate and *Lactobacillus spp.* in probiotic dams), or both, in the juvenile probiotic offspring may have compensated for the creatine energy deficit imposed by maternal obesity (62).

In the adult offspring, gene-expression analysis revealed a long-term effect of maternal probiotic administration on synaptic plasticity. Increased *BDNF* and the immediate early genes *ZIF-268* and *cFOS* indicate an increase in the maintenance of BDNF-mediated synaptic plasticity in adult probiotic offspring (63). Because this mechanism is held to underly response to traditional antidepressant activity and mood resilience in adults (63, 64), it may account for the long-term reduction in depressive-like behavior in these offspring. The elevation of △*FOSB* in the adult offspring of probiotic dams may also contribute to the antidepressant-like behavior, as induced overexpression of △*FOSB* has been shown to counteract the behavioral effects of chronic social defeat stress in mice (65).

To further investigate the etiology of the enduring behavioral and molecular changes in offspring, we explored how maternal obesity and probiotic supplementation affected maternal care behavior (MCB), immunology, and metabolism during nursing. Obesity increased IL-1β expression in dams, whereas probiotic treatment reduced IL-6. In humans, increased maternal IL-6 during pregnancy is predictive of reduced brain connectivity and working memory in 2-y-old offspring (66). Maternal obesity has been directly linked to placental inflammation in NHPs (67), and...
it is conceivable that the divergent maternal proinflammatory activity in obese versus probiotic-supplemented obese and lean dams may have contributed to different neurodevelopment trajectories in the offspring (68).

Maternal probiotic intake increased the abundance of SCFAs in the milk and gut of probiotic-fed dams during nursing, as well as in the gut of their juvenile and adult offspring relative to the vehicle controls in each age group. This may have contributed to the long-term changes in gene expression and behavior exhibited by these offspring. While not detected in brain tissue using NMR, the SCFAs butyrate and propionate are brain-permeable and, like lactate, capable of modulating gene transcription through HDAC modification in vivo (49, 69). We found that increased gut propionate and butyrate coincided with increased anxiety-like behavior in adult offspring. Moreover, these metabolites—gut propionate and butyrate coincided with increased anxiety-like lactate, capable of modulating gene transcription through the SCFAs butyrate and propionate are brain-permeable and, the long-term changes in gene expression and behavior exhibited vehicle controls in each age group. This may have contributed to as in the gut of their juvenile and adult offspring relative to the

Animals. CD-1 IGS mice (Charles River) were housed in a pathogen-free facility under standard conditions. Food and water, including specific dietary administrations, were provided ad libitum. All licensed procedures were performed with UK Home Office approval, under license P99684A4E.

The study design is summarized in Fig. 1A. Dams were a fed an HFD or CD for 12 wk, from 6 wk prior to the start of pregnancy (E0.5) until offspring were of weaning age (PND21). Mice were checked at 08:00 each day for the presence of a vaginal plug. The date of a vaginal plug first appearing was designated E0.5. Males were removed from the cage the following day. Parental (P0) mice were weighed twice a week prior to the start of gestation (Fig. 1B). Offspring (F1) pups were weighed every third day until weaning (SI Appendix, Fig. S14 A and B), and for the adult cohort, weekly thereafter (SI Appendix, Fig. S14 C–E). Probiotic supplementation began at E0.5 and continued until PND21. At this point, dams and half the juvenile offspring were subject to the OFT, LDB, and FST, and tissue was immediately harvested for qPCR (left brain PFC, liver, and fecal) and metabolomics (right brain cerebrum, liver, plasma, and fecal). The remaining offspring were weaned onto regular chow and run through the SIT at 8 wk of age. At PND112, adult offspring were run through the OFT and FST, and tissue was harvested for qPCR and metabolomics.

Treatments. Probiotic administration. Bio-Kult Advanced (ADM Proxin) is a multi-strain probiotic consisting of 14 live strains of bacteria (SI Appendix, Table S34). At E0.5, female F0 mice were randomly assigned to receive either 4 × 107 colony-forming units per milliliter or vehicle only (Fig. 1A). This corresponded to intake of ~6.4 million live bacteria per gram of body weight per day, based on the average daily water consumption of an adult mouse (0.12 ml/kg body mass per day).

Diet. Female F0 mice were randomly assigned to be maintained on either an HFD consisting of 60% kilocalories from fat or a carbohydrate-matched CD with 10% kilocalories from fat (diets from Research Diets Inc.). This resulted in four dams per age group (CD/Vehicle, HFD/vehicle, CD/probiotic, and HFD/probiotic).

Behavior. F0 dams and F1 juvenile mice were subject to an OFT (50 × 50 × 50 cm³, length × width × height [lxwxh]), (LBD, black compartment, 21 × 16 × 16 cm³, lxwxh; white compartment, 46.5 × 21 × 21 cm³, lxwxh), and FST (32 × 17 × 12 cm³, lxwxh) described as described (27). F1 adult mice were tested for social behavior in the SIT (65 × 30 × 20 cm³, lxwxh) at 8 wk of age and the OFT and FST at 16 wk. All behavior was scored while blinded to the identity of treatment groups. Behavioral testing was completed with normal daily environment conditions. Apparatuses were cleaned with 70% ethanol between animals. More detail, including the evaluation of MCB, can be found in SI Appendix.

Tissue Collection. Following behavioral testing, animals were terminally anesthetized under isoflurane gas at the same time each day, to minimize potential circadian variation in metabolic profiles. To collect plasma, blood was collected into lithium heparin-coated tubes via cardiac puncture with a heparinized 23G needle. Blood was left to stand for 30 min at room temperature, then centrifuged at 1,300 × g for 10 min at 4 °C. Plasma lactate was determined with a lactate monitor (EKFDiagnostics). Plasma was stored at −80 °C. Animals were transcardially perfused with cold saline (0.9% NaCl) containing heparin (5,000 USP) until the liver was clear of blood. Whole brain and liver portions were immediately dissected and snap frozen in isopentane. Fecal matter from the distal colon was collected, and all samples were stored at −80 °C until further use.

RNA Extraction, Complementary DNA Conversion, qPCR, and Metabolomic Profiling. Refer to SI Appendix, Methods. List of primers for qPCR can be found in SI Appendix, Table S35.

Statistical Analysis. Statistical tests were performed in Prism 8 (GraphPad) and R (version 3.3.1). Initially, three-way ANOVAs were performed to identify whether sex interacted with maternal diet and/or maternal perinatal probiotic intervention. These processes served to simplify the interpretation of the main effects and their interaction. Prior to three-way or two-way ANOVA, ‘robust’ test for outliers was performed on each group, and statistically significant outliers (P < 0.05) were removed from downstream analyses. Normality of residuals was tested with the Shapiro–Wilks test (P < 0.01). Alternative tests were used for nonparametric data (SI Appendix, Methods). All univariate data were visualized as boxplots showing all data points, where the upper and lower bounds of the box indicate the interquartile range, the line within the box as the median, and the error bars denoting the full range of values. The significance threshold was set at P < 0.05 for behavior and q < 0.05 for univariate metabolomic and Spearman rank correlation analyses adjusted for multiple testing using the FDR method (Benjamini Hochberg).
For offspring gene-expression data, significance was set at \( P < 0.001 \) using the Bonferroni method based on 50 independent tests.

For information on the multivariate analysis performed for the metabolomics data, refer to **SI Appendix, Methods**.

**Data Availability.** All study data are included in the article and/or **SI Appendix**.

The raw metabolomics spreadsheet data have been deposited in the Oxford Research Archive (https://doi.org/10.5287/bodleian:Zo64vBqEn).

**ACKNOWLEDGMENTS.** D.E.R.-S. is supported by the Newtown Abraham Stewardship (Oxford) and the Clarendon Fund in association with the Lincoln College Kinglake award (Oxford).

37. B. A. Pederson et al., Exercise capacity of mice genetically lacking muscle glycogen synthase: In mice, muscle glycogen is not essential for exercise. *J. Biol. Chem.*** 280, 17260–17265 (2005).

38. S. L. Oh et al., Effect of HX108-CS supplementation on exercise capacity and lactate accumulation after high-intensity exercise. *J. Int. Soc. Sports Nutr.* **10**, 21 (2013).

39. M. V. Panajotovic et al., PGC-1α plays a pivotal role in simvatatin-induced exercise endurance. *J. Appl. Physiol.* **118**, e13402 (2020).

40. Y. Niu et al., Pre-gestational intake of Lactobacillus helveticus NS 8 has anxiolytic effects in adolescent Sprague Dawley offspring. Brain Behav. **10**, e01714 (2020).

41. H. M. Navagcic et al., Pre-measurement normalizes lipopolysaccharide (LPS)-induced anxiety and cortical 5-HT2A receptor and 5-HT: β levels in male brain. *Brain Res.** **136**, 202–13 (2006).

42. S. Maeng et al., Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biophy. J.*** **63**, 349–352 (2008).

43. C. A. Zarate Jr et al., A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry*** **63**, 856–864 (2006).

44. R. M. Berman et al., Antidopaminergic effects of ketamine in depressed patients. *Biol. Psychiatry*** **79**, 135–144 (2016).

45. B. Moghaddam, B. Adams, A. Verma, D. Daly, Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J. Neurosci.*** **17**, 2921–2927 (1997).

46. F. Razouk, R. Garcia, I. Lena, Ketamine, at a dose that disrupts motor behavior and cortical theta, reduces hippocampal synaptic efficacy and glutamate release in the nucleus accumbens. *Neuropsychopharmacology** **32**, 719–727 (2007).

47. R. S. Duman, G. K. Aghajanian, G. Sansacora, J. H. Krystal, Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nat. Rev. Neurosci.* **23**, 228–246 (2018).

48. X. Zhang et al., The composition and concordance of lactobacillus populations of infants and the corresponding breast-milk and maternal gut. *Front. Microbiol.* **11**, 597111 (2020).

49. N. Karmi et al., Lactate is an antidepressant that mediates resilience to stress by modulating the hippocampal BDNF system and activity of histone deacetylases. *Neuropsychopharmacology** **44**, 1152–1162 (2019).

50. A. Carrard et al., Peripheral administration of lactate produces antidepressant-like effects. *J. Mol. Psychiatry*** **23**, 1–14 (2018).

51. I. Allaman, H. Fiumelli, P. J. Magistretti, J. L. Martin, Fluoxetine regulates the expression of neurotrophic/growth factors and glucose metabolism in astrocytes. *Psychobiology** **(Berl.)** **216**, 76–84 (2011).

52. L. Pellerin, P. J. Magistretti, Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc. Nat. Acad. Sci. U.S.A.* **91**, 10625–10629 (1994).

53. M. Bélinger, I. Allaman, P. J. Magistretti, Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metab.* **14**, 724–738 (2011).

54. P. Jourdain et al., Dual action of L-Lactate on the activity of NR2B-containing NMDA receptors: From potentiation to neuroprotection. *Sci. Rep.*** **8**, 13472 (2018).

55. J. P. Bolaños, Bioenergetics and redox adaptations of astrocytes to neuronal activity. *Neurochem. Int.*** **139**, 115–125 (2019).

56. K. MacDonald et al., Biomarkers for major depressive and bipolar disorders using metabolomics: A systematic review. *Am. J. Med. Genet. B, Neuropsychiatr. Genet.* **184**, 123–137 (2018).

57. A. V. Bakian, R. S. Huber, L. Scholl, P. F. Renshaw, D. Kondo, Dietary intake and depression risk among U.S. adults. *Transl. Psychiatry** **10**, 52 (2020).

58. D. D. Condi et al., Overweight female adolescents with SRI-resistant major depressive disorder: A 31-phosphorus magnetic resonance spectroscopy study. *J. Affect. Disord.** **135**, 354–361 (2011).

59. D. G. Harper et al., Tissue-specific bioenergetic abnormalities in adults with major depression. *Neuropsychopharmacology** **42**, 876–885 (2017).

60. F. P. Della et al., Tianeptine treatment induces antidepressant-like effects and alters BDNF and energy metabolism in the brain of rats. *Behav. Brain Res.*** **233**, 526–535 (2012).

61. X. Song et al., Bioenergetics and abnormal functional connectivity in psychiatric disorders. *Mol. Psychiatry*** **26**, 2483–2492 (2015).

62. C. Stork, E. Castrén, T. Rantamäki, The role of BDNF and its receptors in depression and anxiolytic effects of strontial ΔFo overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol. Psychiatry*** **70**, 550–558 (2014).

63. E. Castrén, T. Rantamäki, The role of BDNF and its receptors in depression and anxiolytic effects of strontial ΔFo overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol. Psychiatry*** **70**, 550–558 (2014).

64. R. J. Donohue, J. W. Muschamp, S. J. Russo, E. J. Nester, W. A. Carlezon Jr, Effects of strontial ΔFo overexpression and ketamine on social defeat-stress induced anhedonia in mice. *Biol. Psychiatry*** **70**, 550–558 (2014).

65. M. D. Rudolph et al., Maternal IL-6 during pregnancy can be estimated from newborn brain connectome and predicts future working memory in offspring. *Nature Neurosci.** **21**, 765–772 (2018).

66. A. E. Fries et al., Maternal high-fat diet disturbs uteroplacental hemodynamics and increases the frequency of stillbirth in a nonhuman primate model of excess nutrition. *Endocrinology** **152**, 2456–2464 (2011).

67. J. W. van der Burg et al., The role of systemic inflammation linking maternal BMI to placental and fetal development in a pig model of maternal obesity. *Front. Physiol.*** **9**, 1–14 (2018).

68. N. Arpaia et al., Metabolites produced by commensal bacteria promote peripheral inflammation. *Nature*** **504**, 451–455 (2013).

69. D. G. Covian et al., Increased maternal dietary acids and their link with diet and human health. *Front. Microbiol.* **8**, 1 (2017).

12 of 12 | **PNAS**

Modifying the maternal microbiota alters the gut-brain metabolome and prevents emotional dysfunction in the adult offspring of obese dams.