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Gut microbiota signatures of vulnerability to food addiction in mice and humans

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ABSTRACT

Objective Food addiction is a multifactorial disorder characterised by a loss of control over food intake that may promote obesity and alter gut microbiota composition. We have investigated the potential involvement of the gut microbiota in the mechanisms underlying food addiction.

Design We used the Yale Food Addiction Scale (YFAS) 2.0 criteria to classify extreme food addiction in mouse and human subpopulations to identify gut microbiota signatures associated with vulnerability to this disorder.

Results Both animal and human cohorts showed important similarities in the gut microbiota signatures linked to food addiction. The signatures suggested possible non-beneficial effects of bacteria belonging to the Proteobacteria phylum and potential protective effects of Actinobacteria against the development of food addiction in both cohorts of humans and mice. A decreased relative abundance of the species *Blautia wexlerae* was observed in addicted humans and of *Blautia* genus in addicted mice. Administration of the non-digestible carbohydrates, lactulose and rhamnose, known to favour *Blautia* growth, led to increased relative abundance of *Blautia* in mice faeces in parallel with dramatic improvements in food addiction. A similar improvement was revealed after oral administration of *Blautia wexlerae* as a beneficial microbe.

Conclusion By understanding the crosstalk between this behavioural alteration and gut microbiota, these findings constitute a step forward to future treatments for food addiction and related eating disorders.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Food addiction, characterised by loss of control over food intake, may promote obesity and alter gut microbiota content.
⇒ Brain alterations related to behavioural disorders have been reported to modify the gut microbiome, and gut microbes alter the neurobiology of brain regions involved in behavioural control.

WHAT THIS STUDY ADDS

⇒ We have identified specific gut microbiota content associated 'in our human and animal cohorts' with the differential vulnerability to develop food addiction.
⇒ We have also functionally validated in animals the beneficial role of *Blautia* and rhamnose in preventing the development of the behavioural hallmarks of food addiction.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The elucidation of the specific microbiota content associated with food addiction may provide new biomarkers for this behavioural disorder.
⇒ The identification of these novel biological mechanisms provides new advances toward innovative interventions for food addiction and related disorders using beneficial microbes and/or dietary supplementation.

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INTRODUCTION

The gut microbiota integrates a large variety of microbes, including bacteria, fungi, archaea and viruses that colonise the digestive tract. These microorganisms offer numerous benefits by interacting with the host and establishing a symbiotic relationship.[1] This symbiosis is not only essential for peripheral physiological functions, but also affects neurobiological processes. Multiple studies have demonstrated the existence of a crosstalk between several neurobiological processes and the gut microbiota, including those related to mental disorders, such as depression,[2] anxiety, autism spectrum disorders and addiction.[2–4] Interestingly, the relationships between addiction and dysbiosis in gut microbiota are gaining high relevance, mainly in alcohol abuse.[5] Ethanol consumption reduces protective bacteria, increases intestinal permeability

and releases inflammatory factors, which finally contribute to the psychopathology of alcoholism.[6] Other substance use disorders, such as opioid, cocaine or methamphetamine disorders, have also been related to gut microbiota dysbiosis.[7]

Food addiction is a controversial concept still under debate,[8] consisting of a complex multifactorial behavioural disorder characterised by a loss of control over food intake that has increased in prevalence in recent years.[9] It is characterised by the compulsive intake of palatable foods, which can produce adaptive changes in the reward brain network. This behavioural alteration is related to obesity and other eating disorders and lacks effective treatment, leading to high socioeconomical costs worldwide. Despite the early definition of this concept,[10] the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) does not include the concept of food addiction.[11] However, a widely accepted instrument currently used in the clinic to evaluate food addiction is the Yale Food Addiction Scale (YFAS), updated in 2016 to YFAS 2.0 to apply the DSM-5 criteria for substance use disorder to food addiction.[12] The YFAS 2.0 food addiction criteria can be summarised in three hallmarks also used in rodent models to mimic this disorder: persistent food seeking, high motivation to obtain food, and compulsivity-like behaviour.[13 14] In previous studies, we have validated the animal model of food addiction in mice,[15] and these behavioural findings were replicated in other studies.[13 14] We found that the specific prelimbic to the nucleus accumbens brain circuit was involved in the vulnerability to develop food addiction, and we have also described specific epigenetic mechanisms involved in this multifactorial disease.[13 14]

The study of the gut microbiota signatures related to food addiction has gained attention in recent years. However, most studies have been performed in rodents,[16–18] and few human studies have been reported.[19] In spite of these findings, there is a lack of translational studies that validate the functional relevance of human findings in animal models, which would be required to design more successful treatments.[8] Environmental factors and dietary patterns have a major influence on gut microbiota composition, and the overconsumption of highly palatable food may promote a gut microbiota dysbiosis that has been recently proposed to participate in the loss of eating control.[8 19] In agreement, individuals with obesity, which may be promoted by food addiction,[20] showed altered gut microbiota with a reduced diversity that facilitated energy absorption capacity and may affect host brain function.[21] However, the functional relevance of gut microbiota in the loss of eating control and food addiction has not yet been demonstrated.

In this study, we have obtained extreme subpopulations of food addicted and non-addicted mice to identify the differential gut microbiota signatures associated with vulnerability to addiction. Using parallel food addiction-like criteria, we have applied the YFAS 2.0 score to classify a cohort of patients to assess the possible gut microbiota signatures of this behavioural disorder as potential biomarkers. We functionally validated the role of *Blautia*, the genus most differentially expressed in addicted mice and humans, by administering the non-digestible carbohydrates,[22] lactulose and rhamnose, that increased *Blautia* abundance and prevented the development of food addiction in mice. A similar result was observed after oral administration of *Blautia wexlerae* as a beneficial microbe. The strategy of a beneficial microbe and/or dietary supplements to modulate gut microbiota is promising and can be either extracted from non-digestible carbohydrate materials or synthetically produced.[23]

METHODS

Detailed methods are provided in the online supplemental material.

RESULTS

Characterisation of extreme subpopulations of addicted and non-addicted mice

We used the genetically homogeneous inbreed C57Bl/6J strain of mice that underwent an operant protocol of food addiction during six sessions of fixed ratio (FR) 1 schedule of reinforcement, followed by 92 daily sessions of FR5 (figure 1A). The selected subset of mice belongs to a previous publication with a large cohort of male JAX C57BL/6J mice to evaluate miRNA signatures associated with vulnerability to food addiction.[14] In the late period of the food addiction protocol (figure 1A), addicted mice performed higher persistence of response, motivation and compulsivity than non-addicted mice, as expected (figure 1B–D). In contrast, intake of pellets and body weight were similar in addicted and non-addicted mice (figure 1E, F), possibly due to the limited effort (FR5) required to obtain each reward. Online supplemental material and online supplemental table S1 provide a detailed description of the results of figure 1.

Gut microbiota profile of vulnerability to addiction in mice

We carried out the 16S rRNA gene amplicon sequencing of caecum contents to study gut microbiota signatures associated with food addiction-like behaviour using the cohorts of extreme phenotypes described above. As expected, the murine caecal microbiota was dominated by the phyla Firmicutes and Bacteroidetes, reaching almost 90% of the relative abundance, similar in both addicted and non-addicted mice (figure 2A). See online supplemental material for a detailed description of the results of figure 2. Alpha diversity indexes were similar in both groups (figure 2B, C), and there were no differences in beta diversity (online supplemental figure S1). Importantly, analyses of the data at the different taxonomic levels showed significant differences in the phyla, families and genera's relative abundances of several bacteria. Actinobacteria phylum (figure 2D), Coriobacteriaceae and Erysipelotrichaceae families (figure 2E), and Lachnospiraceae UCG-001 and Enterohabdus genera (figure 2F) had decreased relative abundances in addicted compared with non-addicted mice. Other genera, such as Allobaculum and *Blautia* (figure 2F) from Bacillota/Firmicutes phylum, showed a similar decrease in addicted mice, altogether supporting the potential beneficial profile of non-addicted gut microbiota signatures.

Gut microbiota correlates with food addiction features in mice

The possible correlations between gut microbiota signatures, addiction-like criteria, and the phenotypic traits of the different mice groups were further investigated (figures 3 and 4, online supplemental figure S2, online supplemental figure S3). At the genus level (figures 3 and 4), the Ruminococcaceae_NK4A214_group and the Gastranaerophilales_uncultured organism genera positively correlated with motivation in the addicted group, whereas the Clostridiales_vadin BB60 group_uncultured, Erysipelatoclostridium and Parabacteroides genera negatively correlated with motivation in these addicted mice. Finally, the genera Ruminococcaceae_UCG009, Lachnospiraceae_FCS020_group, Peptococcus, Candidatus_Arthromitus, Ruminiclostridium_6, Roseburia, Coprococcus_1 and Acetatifactor positively correlated with persistence of response in the addicted group. Online supplemental material has a detailed description

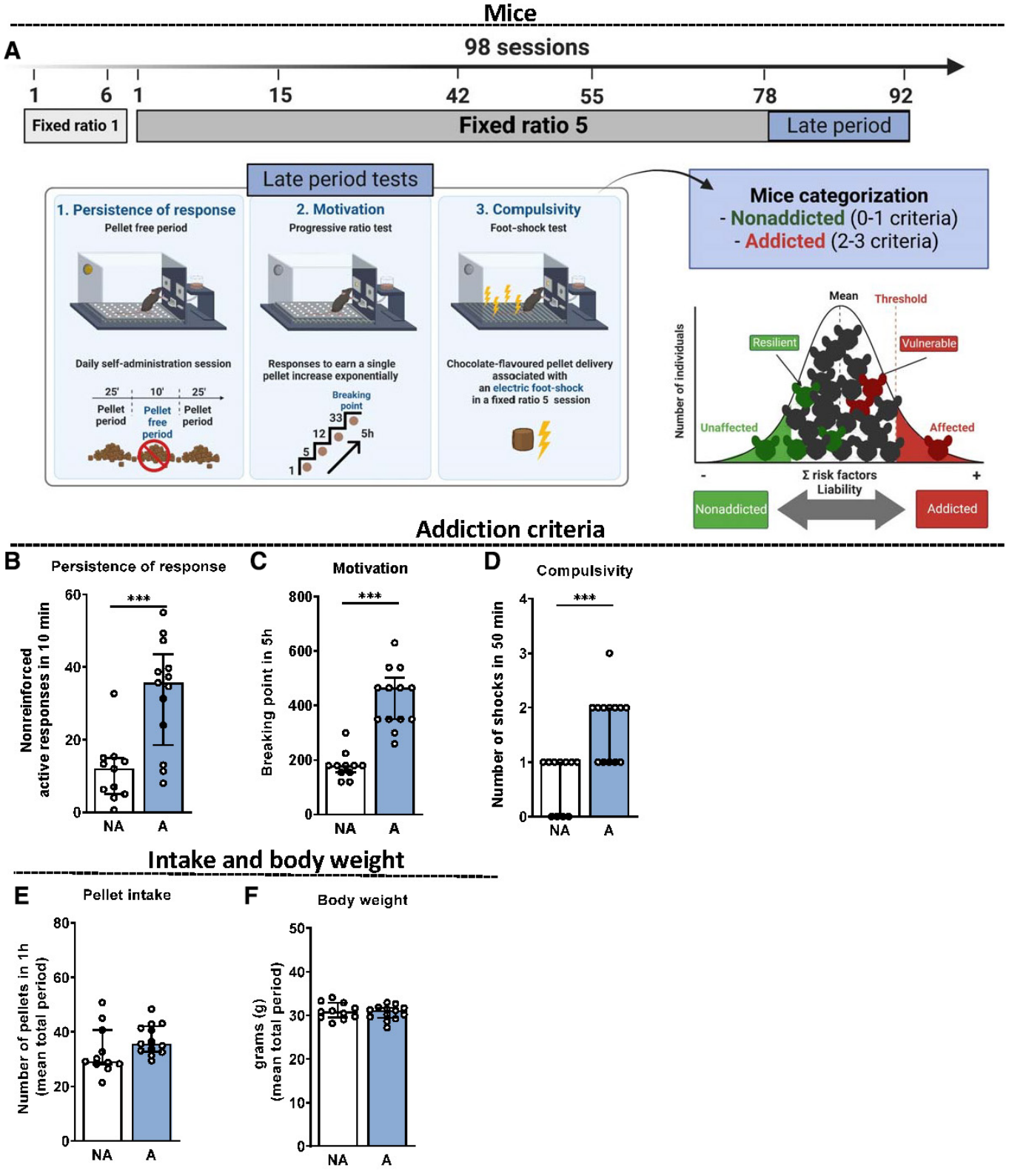

Figure 1 Characterisation of extreme subpopulations of addicted and non-addicted mice. (A) Timeline of the procedure of operant behaviour mouse model. Mice were trained during the first 6 days in operant behaviour sessions of 1 hour at a fixed ratio (FR) 1 schedule of reinforcement, followed by 92 daily sessions of FR5. The addiction-like criteria (persistence of response, motivation and compulsivity) were evaluated in the late period to categorise mice as addicted and non-addicted. (B–D). Behavioural tests for the three addiction-like criteria in the late period (individual values with IQR) in the addicted and non-addicted groups. (B) Persistence of response (t test, ***p<0.001). (C) Motivation (Mann–Whitney U test, ***p<0.001). (D) Compulsivity (Mann–Whitney U test, ***p<0.001). (E) Pellet intake and (F) body weight for those mice classified as addicted (A) and non-addicted (NA) (n=11 mice as A and n=13 as NA mice, trained with chocolate pellets). Statistical details are included in online supplemental table S1. The selected subset of mice belongs to a previous publication with a large cohort of male JAX C57BL/6 J mice to evaluate miRNA signatures associated with vulnerability to food addiction.[14]

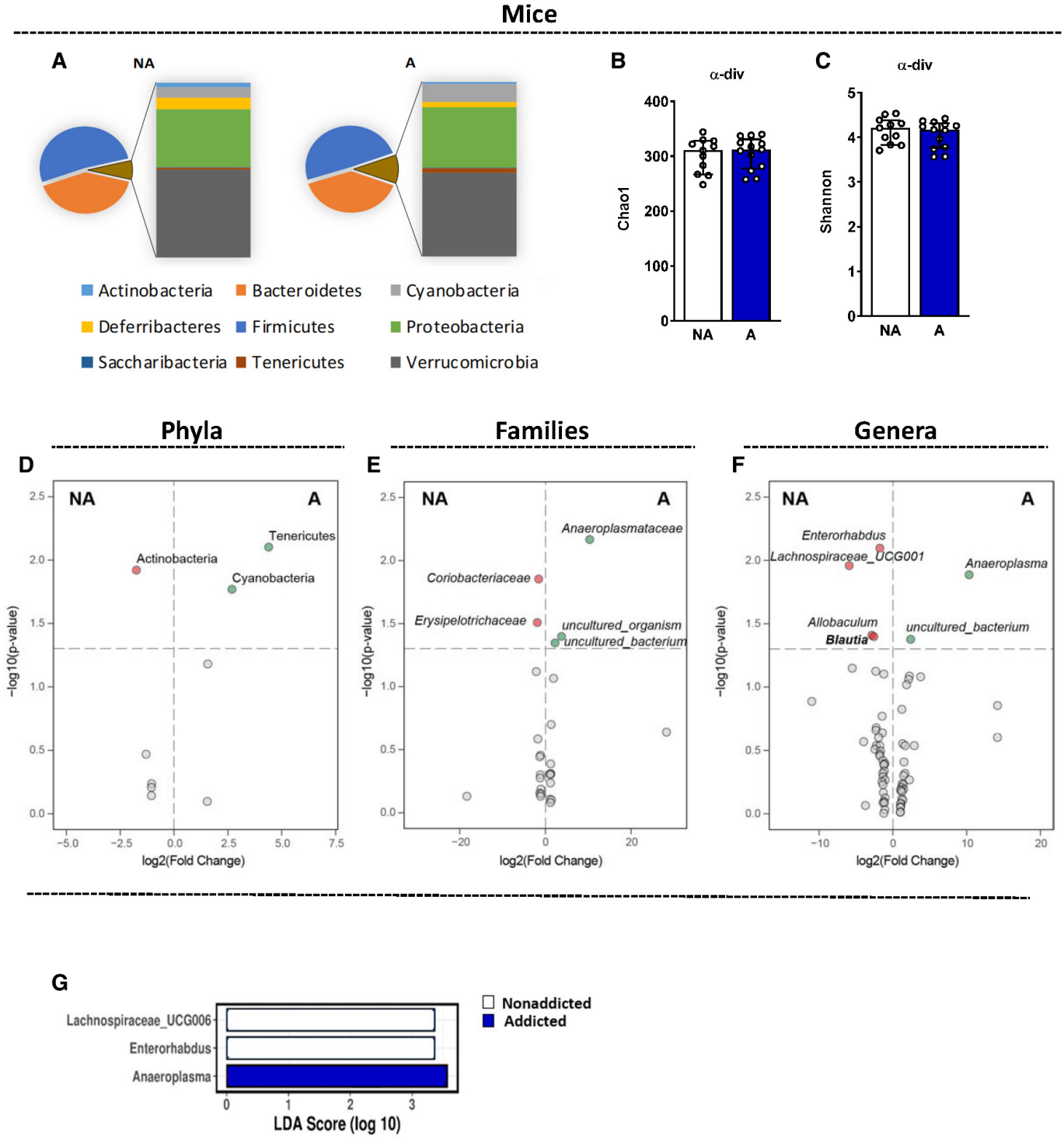

Figure 2 Gut microbiota profile of vulnerability to addiction in mice. (A) Pie chart at the phylum level shows a high proportion of the phyla Firmicutes and Bacteroidetes, reaching almost 90% of the relative abundance in both groups of addicted and non-addicted mice. (B–C) Results of Chao1 and Shannon alpha diversity indexes of addicted (A) and non-addicted (NA) mice. (D–F) Volcano plots representing the differential bacterial abundance between addicted (A) and non-addicted (NA) mice after a long operant training protocol using the DESeq^2 test. Differences were observed in the relative abundances at the (D) phylum, (E) family and (F) genus levels. The volcano plot indicates −log 10 (p value) for bacteria (Y axis) plotted against their respective log 2 (fold change) (X axis). The coloured dots represent significantly downregulated and upregulated bacteria between the addicted and non-addicted groups, respectively (ie, *Blautia* is downregulated in addicted mice). Significantly different taxa ($p<0.05$) are coloured according to the phylum. (G) Discriminant analysis effect size method (LEfSe) comparing addicted and non-addicted groups. Linear discriminant analysis (LDA) was performed. Data are expressed as mean±SEM. DESeq^2 test wa performed (n=11 non-addicted (NA) mice, n=13 addicted (A) mice).

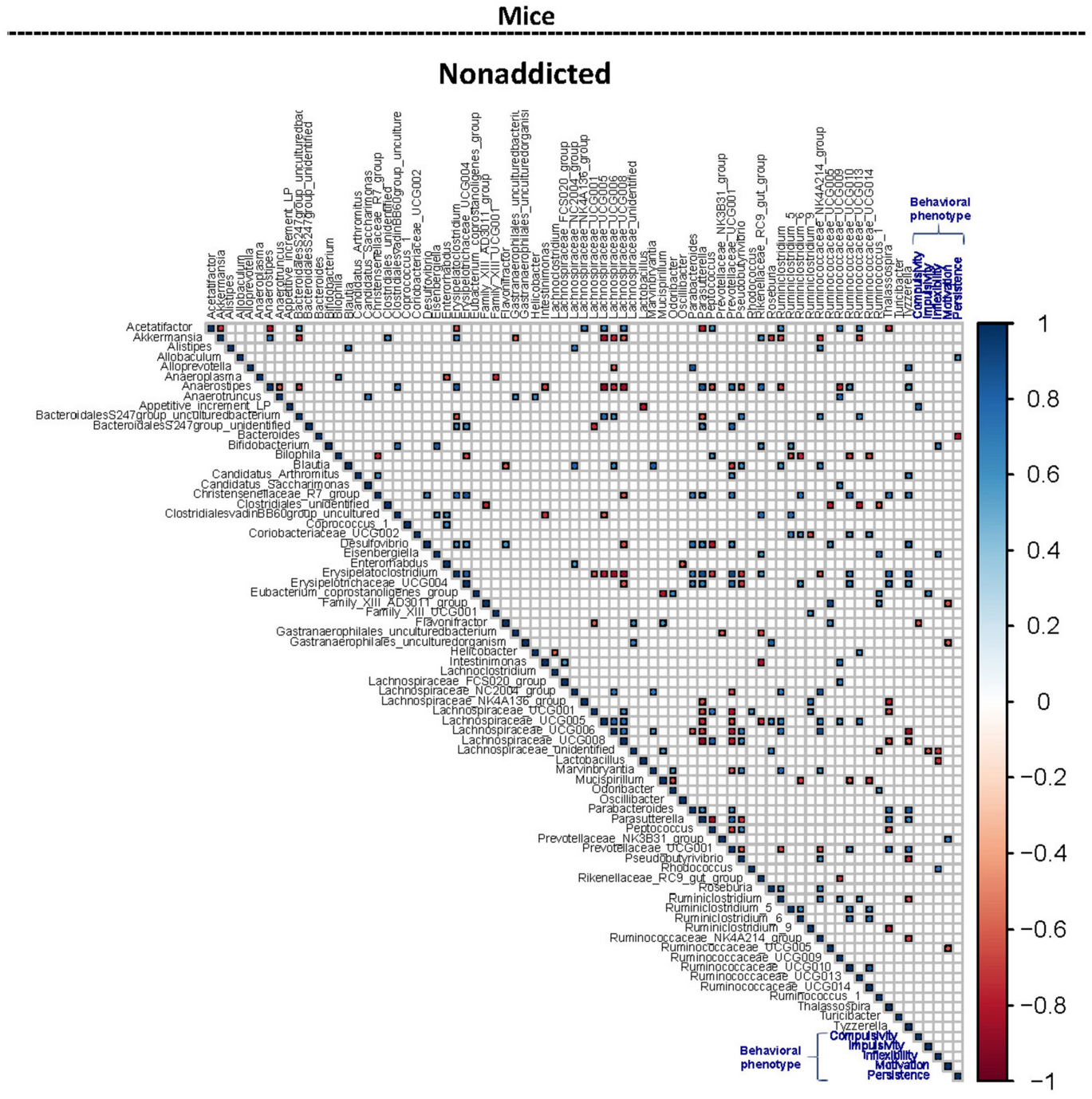

Figure 3 Caecal microbiota of non-addicted mice. Corrplot showing significant (p<0.05) Spearman correlations coefficients between microbiota relative abundances at genus level and addiction criteria and phenotypic traits in non-addicted mice after a long operant training protocol (n=11 for non-addicted mice).

of the results of microbiota and food addiction signature correlations at the family level (online supplemental figure S2 and S3).

Use of YFAS 2.0 to characterise addicted and non-addicted humans

The possible gut microbiota signatures associated with food addiction were investigated in a cohort of patients (n=88) classified following the YFAS 2.0 questionnaire. The three addiction-like criteria measured in our food addiction mouse model recapitulates properly the principal features of the food addiction human disease evaluated by the 35 item self-report YFAS 2.0, as reported previously.[14] As expected, the sum of the YFAS 2.0 questions, under the criteria of persistence of response, motivation and compulsivity, was much higher in participants diagnosed with food addiction than in non-addicted subjects (figure 5A–C). We also performed a principal component analysis (PCA) with the main variables (persistence of response, motivation, compulsivity, tolerance, withdrawal, craving and distress). The two principal components (PC) accounted for 75.8% and 7.1% of variations, respectively (figure 5D–G), and two clusters of addicted and non-addicted groups were identified (figure 5D). Interestingly, PC1 has strong loadings (>0.7) from

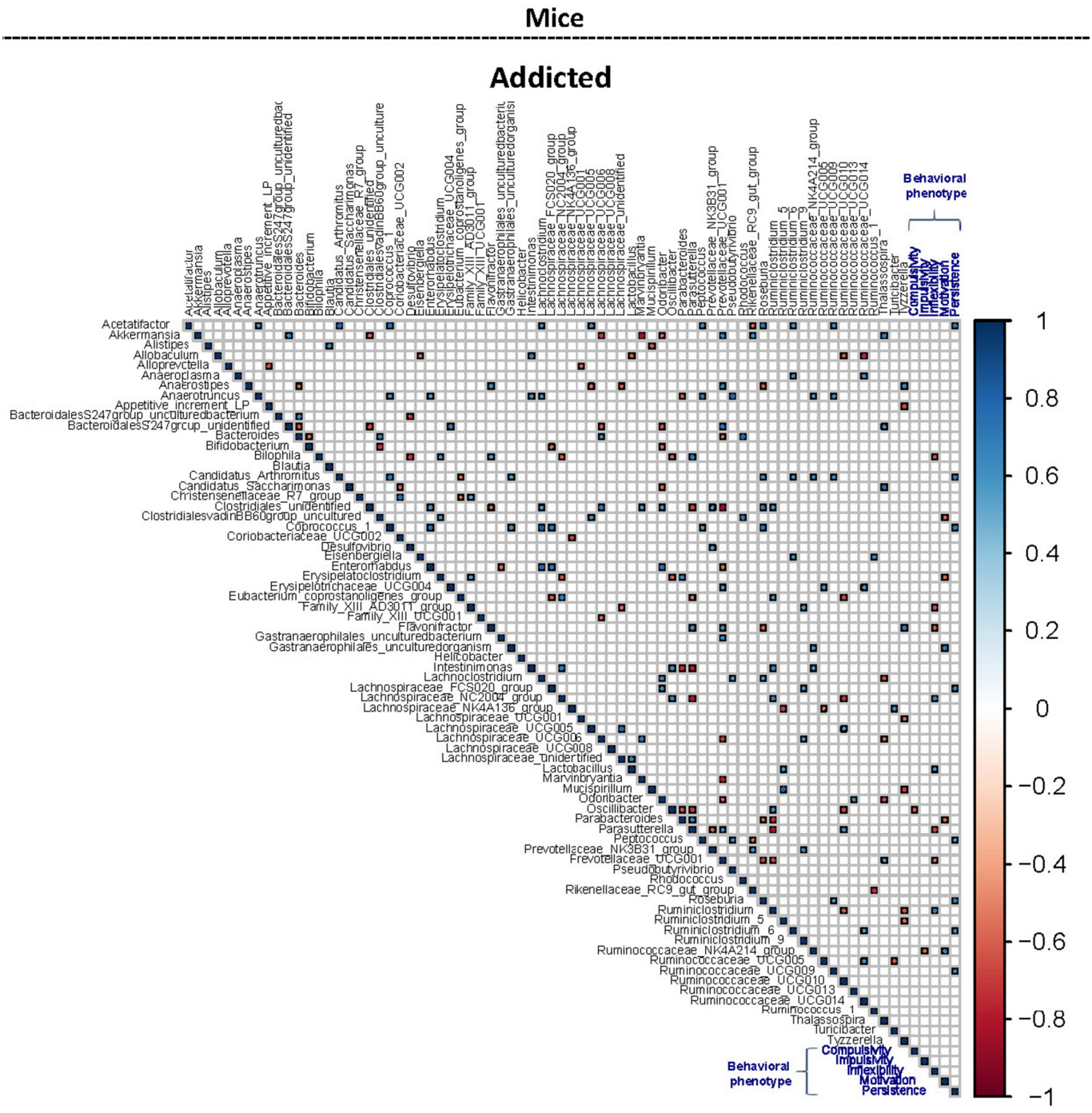

Figure 4 Caecal microbiota of addicted mice. Corrplot showing significant ($p<0.05$) Spearman correlations coefficients between microbiota relative abundances at genus level and addiction criteria and phenotypic traits in addicted mice after a long operant training protocol (n=13 for addicted mice).

distress, withdrawal, tolerance and craving (figure 5E, F) but not from the persistence of response, motivation or compulsivity that had high loads in PC2 (figure 5E, G). Online supplemental material has a detailed description of the results of figure 5D–G.

Cross-characterisation of mouse and human behaviours

Correlational analyses between addiction criteria in mice and humans were also plotted together (figure 5H–J). The links between correlations found in mice and humans further underlined the translational value of our behavioural results. In non-addicted individuals, a positive correlation was found in both humans and mice between the following addiction criteria: compulsivity and persistence of response (figure 5H), compulsivity and motivation (figure 5I), and motivation and persistence of response (figure 5J). We also performed a correlation matrix to explore the nature of the association between each addiction criterion and each phenotypic trait with the cohorts of mice and humans, respectively (online supplemental figure S4). Online supplemental material has a detailed description of the results of figure 5 and online supplemental figure S4.

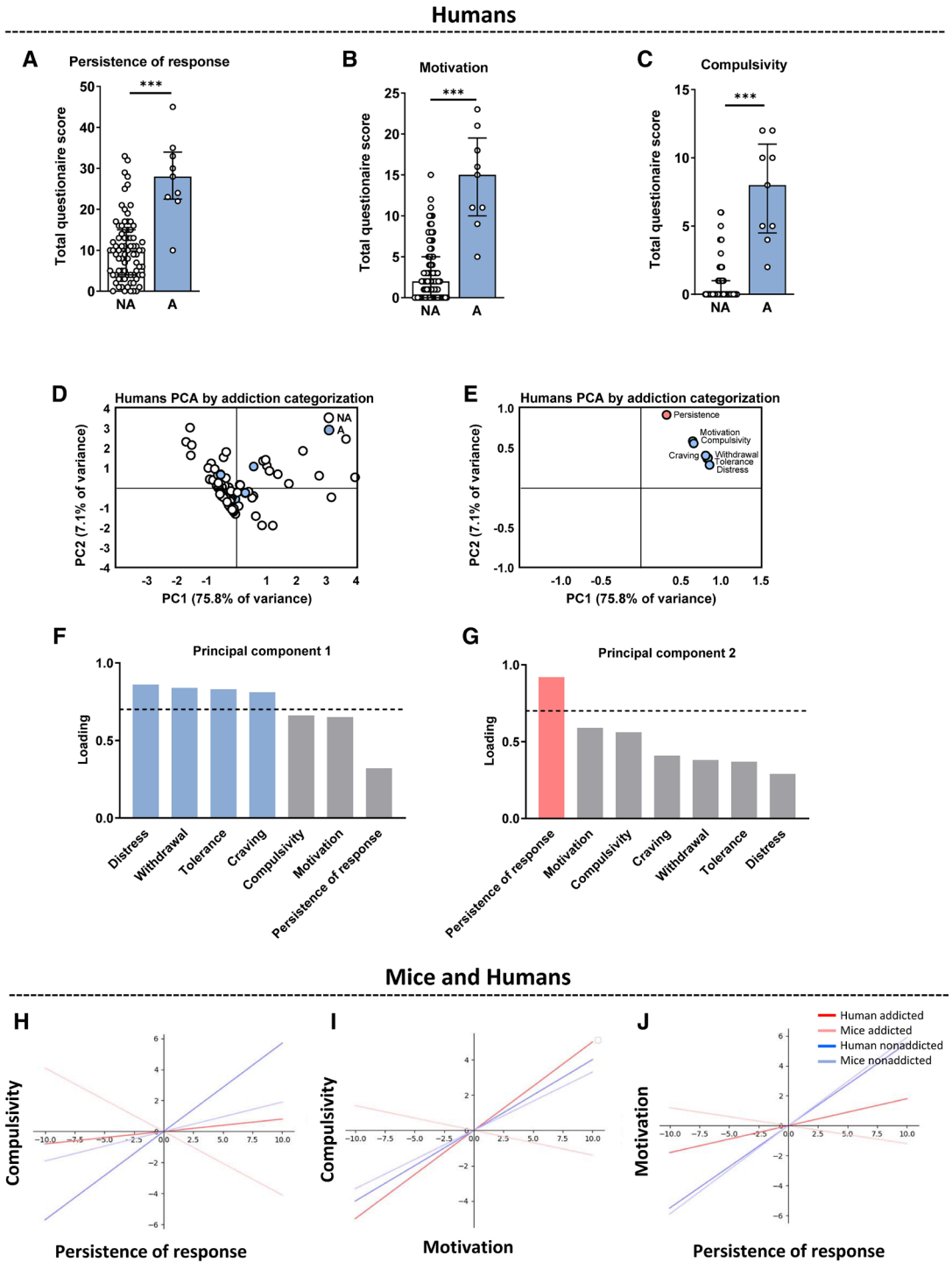

Figure 5 Gut microbiota signatures in humans. (A–C) Results of the three addiction-like criteria. (A) Persistence of response (Mann–Whitney U test, ***p<0.001), (B) motivation (Mann–Whitney U test, ***p<0.001), and (C) compulsivity (Mann–Whitney U test, ***p<0.001) (median and IQR), comparing non-addicted (NA) and addicted (A) participants. n=88 for human participants (n=79 NA individuals, n=9 A individuals). (D–G) Principal component analysis (PCA) of the three addiction criteria and the four phenotypic traits in humans. (D) Human subjects clustered by addicted or non-addicted classification on the space yielded by two components of the PCA that account for the maximum data variance. (E) Criteria belonging to each component, principal component (PC) 1 (75.8%) and PC2 (7.1%). (F, G) The order of factor loading of the different variables in PC1 and PC2 is represented. The dashed horizontal line marked loadings >0.7, mainly contributing to the component. (H–J) Correlational analyses between addiction criteria of persistence of response, motivation and compulsivity in addicted and non-addicted mice compared with addicted and non-addicted individuals belonging to the human cohort were analysed together. (H) At the correlation between compulsivity and persistence of response, a strong negative correlation for addicted individuals (both human and mice) and a moderate positive correlation for non-addicted individuals were described. (I) At the correlation between compulsivity and motivation, a mild negative correlation for addicted individuals (mice) was found, while positive correlations for non-addicted individuals (mice and humans) and addicted humans were described. (J) At the correlation between motivation and persistence of response, a strong negative correlation for addicted individuals (humans) and mild for mice was found together with a moderate positive correlation for non-addicted individuals (mice and humans) with a similar slope.

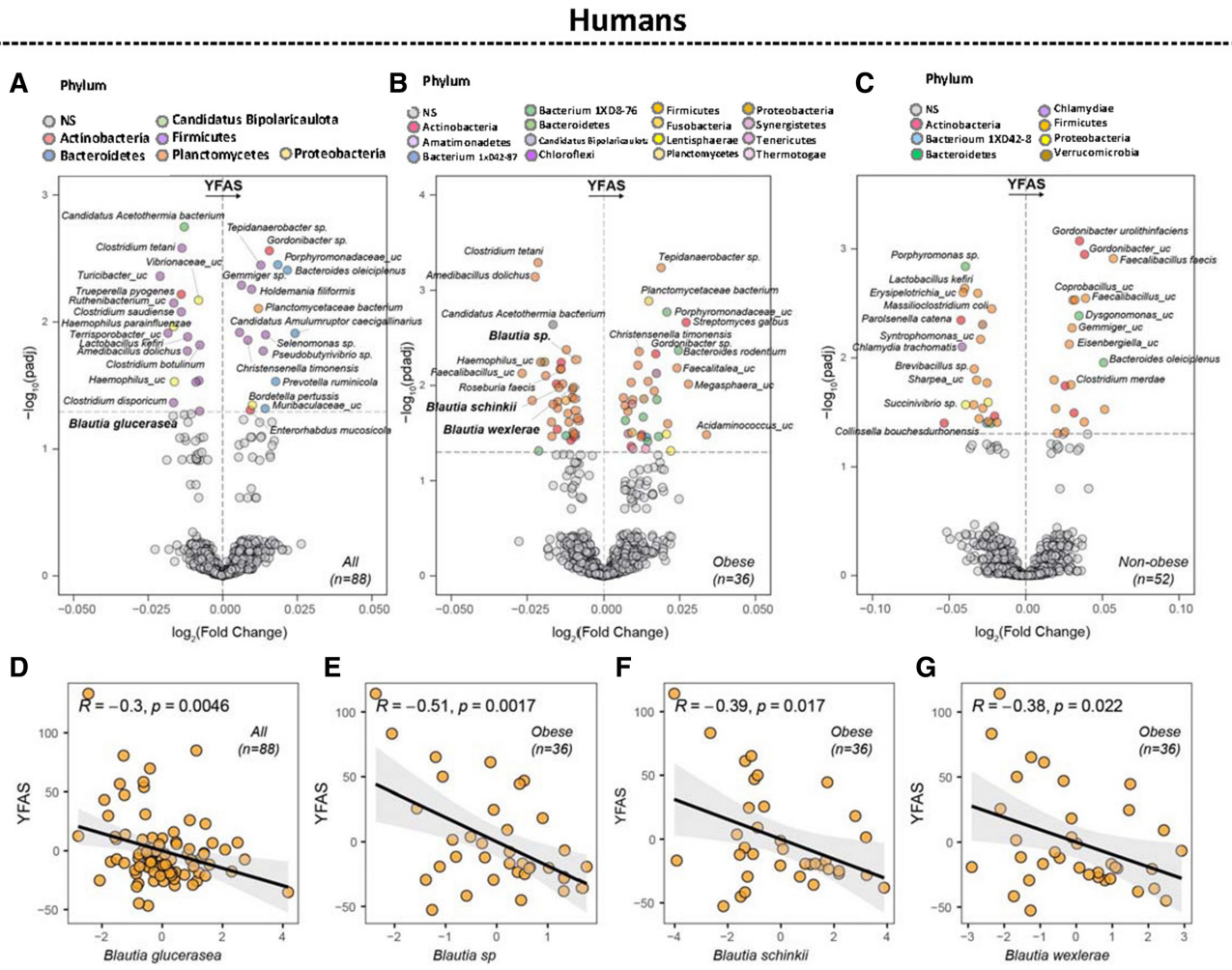

Figure 6 Volcano and scatter plots of bacterial abundance. (A–C) Volcano plots representing the differential bacterial abundance (pFDR<0.05) using ANCOM-BC, controlling for age, body mass index and sex in humans for (A) all of the population (n=88), and (B) obese (n=36) and (C) non-obese (n=52) individuals. Fold change (FC) associated with a unit change in the YFAS score and log10 Benjamini–Hochberg p values adjusted (pFDR) are plotted for each taxon. Significantly different taxa are coloured according to the phylum. NS, non-significant. (D–G) Scatter plots of the partial Pearson correlation between the centred log ratio (clr) levels of different species of the genus *Blautia* and the Yale Food Addiction Scale (YFAS) scores in (D) the whole cohort (n=88) controlling for age, body mass index and sex, and in (E–G) patients with obesity (n=36), controlling for age and sex. The residuals are plotted.

Gut microbiota signatures in humans

Differential bacterial abundance using ANCOM-BC, controlling for age, body mass index and sex were observed in our human cohort (n=88) in the volcano plots (figure 6A–C). Microbiota signatures, distinct between addicted and non-addicted individuals, are represented by the differential expression of bacteria species taxa coloured according to the phylum (figure 6A). Multiple similitudes were found to be overlapped between mice and humans. Interestingly, *Blautia wexlerae* species were increased in individuals with a low score in the YFAS scale in obese humans (figure 6B), in agreement with the decreased *Blautia* genus abundance in addicted mice (figure 2F). Additionally, there were no differences in beta diversity (online supplemental figure S5). We explored the influence of diet composition on the gut microbiota data and did not observe any effect (online supplemental table S2). Online supplemental material has a detailed description of the results of figure 6A–G.

Functional validation with the non-digestible carbohydrates lactulose and rhamnose in the mouse food addiction protocol

Both mice and human results suggest that some specific microbiota could be protective in preventing food addiction. The strong similitudes found in the *Blautia* genus content in both species underlines the potential beneficial effects of this particular gut microbiota. However, *Blautia* is a strictly anaerobic bacteria, and its possible therapeutic use as a a beneficial microbe to prevent food addiction would be difficult. Interestingly, several well known prebiotics that could be used in humans have been reported to enhance the abundance of the *Blautia* genus. Therefore, we have investigated the possible protective effects promoted by oral administration of lactulose and rhamnose as non-digestible carbohydrates able to enhance *Blautia* genus abundance in the gut.[24 25]

For this purpose, a total of 41 C57BL/6J mice underwent an operant protocol of 120 sessions (6-FR1 and 114-FR5 sessions (online supplemental figures S6 and S7 and online supplemental table S3). Online supplemental material has a detailed

description of the behavioural results of figure 7 and the PCA and correlation matrix of figure 8. Importantly, motivation for chocolate flavoured pellets was significantly reduced in the rhamnose group compared with lactulose (Mann–Whitney U test=31.50, p<0.05, figure 7C). Significantly, mice receiving rhamnose showed decreased compulsivity in seeking palatable food compared with control mice, revealing a beneficial effect of this non-digestible carbohydrate to prevent food addiction (Mann–Whitney's U=49.50, p<0.05, figure 7D). Notably, 29.41% of control mice achieved 2–3 criteria and were considered addicted, whereas none of the mice receiving lactulose or rhamnose achieved the addiction criteria (χ^2=5, control vs lactulose p<0.05 and χ^2=5, control vs rhamnose p<0.05, figure 7E).

We next analysed the faecal microbiota composition of mice from the functional validation experiment after oral administration of lactulose and rhamnose. We found that supplementation with rhamnose increased the levels of several species from the SCFA producing family Lachnospiraceae, including several species from the genus *Blautia*, such as *Blautia faecis*, *Blautia* sp, *and Blautia_uc* (online supplemental figure S7A). Online supplemental material has a detailed description of the microbiota composition results shown in online supplemental figure S7.

Functional validation with the beneficial microbe *Blautia wexlerae* in the mouse food addiction protocol

To further demonstrate that *Blautia* has a protective effect against the development of food addiction, oral administration of *Blautia wexlerae* in mice that underwent the long protocol of food addiction (6-FR1 and 114-FR5 sessions) described in the previous section was performed. We used the same regimen of chronic *Blautia* oral administration 3 days per week at a concentration (1×10^9 CFU) similar to what was described before to prevent obesity and type 2 diabetes.[26] For this purpose, a total of 37 C57BL/6J mice underwent an operant protocol of 120 sessions (figure 9). Online supplemental material has a detailed description of the behavioural results shown in figure 9.

Importantly, *Blautia* treated mice showed similar persistence of response, but significantly reduced motivation, and decreased compulsivity for highly palatable food compared with control mice, revealing a protective effect of *Blautia* to develop food addiction (Mann–Whitney U test, p<0.05, figure 9B–D, online supplemental table S4). Remarkably, 21.05% of control mice reached 2–3 addiction criteria in the late period (98–114 sessions), whereas none of the mice receiving *Blautia wexlerae* were classified as addicted (χ^2=4.80, control vs *Blautia* p<0.05, figure 9E). No significant differences between groups were found in impulsivity, cognitive inflexibility, appetitive cue reactivity and aversive cue reactivity (figure 9F–I). In addition, all mice had similar food intake and body weight during the experimental sequence (figure 9J, K).

PCA and correlation heatmap were performed to understand further the correlation between *Blautia* administration and behavioural phenotypes leading to the prevention of food addiction. Online supplemental material has a detailed description of the results shown in figure 10.

Blautia correlates with phenotypic features in mice and humans

Correlational analyses were performed between *Blautia* and the phenotypic traits in humans and mice. In human studies, *Blautia* correlated negatively with distress, and in mouse studies, *Blautia* correlated negatively with the number of addiction criteria. We have included this information in online supplemental figure S8 and the description of statistical analyses in online supplemental material.

qPCR gene expression from the functional validation with *Blautia* treatment in the mouse food addiction protocol

No significant differences in gene expression were obtained between mice treated with *Blautia* or vehicle when evaluating four main targets of the dopaminergic system by qPCR in the key regions of the reward system such as the medial prefrontal cortex (mPFC) or the nucleus accumbens (NAc): *Drd2* (dopamine receptor type 2), *Drd1* (dopamine receptor type 1), *Th* (tyrosine hydroxylase) and *Darpp32* (dopamine and cAMP regulated neuronal phosphoprotein). Online supplemental figure S9 and online supplemental table S5 have more details.

DISCUSSION

We have identified a particular gut microbiota signature in mice and humans with relevant changes in specific microbiota phyla, families and genera when comparing addicted and non-addicted phenotypes after a long operant training in the former, despite similar pellet intake, identical diet, and experimental and housing conditions, even considering that diet is the primary modulator of gut microbiota.[27] Remarkably, animals reaching food addiction criteria showed lower abundances than non-addicted mice in several important bacterial groups, including *Enterorhabdus*, *Lachnospiracceae*, *Allobaculum* and *Blautia* genera, suggesting potential protective effects. In contrast, the relative abundance of *Anaeroplasma* genus *and Gastranaerophilales* families were increased in addicted mice, suggesting non-beneficial effects. This differential microbiota profile is relevant considering their relative abundance and the previous literature associating these bacteria with the gut–brain axis.[28 29] These associations were correlational and suggested a probable bidirectional communication between the gut and the brain, where the gut can connect with the brain through hundreds of metabolites, among others, and the brain can link with the gut via autonomic nervous system signalling that regulates gut bacterial abundance.[8]

Our results suggest that an increased relative abundance of *Enterorhabdus* genus (Actinobacteria phylum) in the gut of non-addicted mice can be beneficial, in agreement with the previous literature. Although *Enterorhabdus* levels have not been previously associated with addiction, previous studies revealed a beneficial effect on other psychiatric disorders. Negative correlations were also observed between *Enterorhabdus* and brain kynurenine levels of mice that underwent chronic mild stress, with increased kynurenine levels being a reflection of disturbances in tryptophan metabolism.[30] In agreement with the results in mice, the species from the Actinobacteria phylum (*Bifidobacterium dentium*) were upregulated in our non-addicted individuals with low YFAS 2.0 scores. The coincidence at the phylum level in mice and humans can help to identify specific candidates to predict loss of eating control across organisms, improving the translatability of our work, mainly considering that only a 10% overlap occurs at the species level between mouse and human gut microbiota profiles.[31]

Our results in mice also showed a potential beneficial effect of *the Lachnospiraceae* genus from the Bacillota/Firmicutes phylum in food addiction that was coherent with the results obtained in humans for several species of the phylum to which it belongs. Previous studies showed that the abundance of the *Lachnospiraceae* genus was modified in a forced alcohol drinking group of mice. However, the results were controversial, suggesting

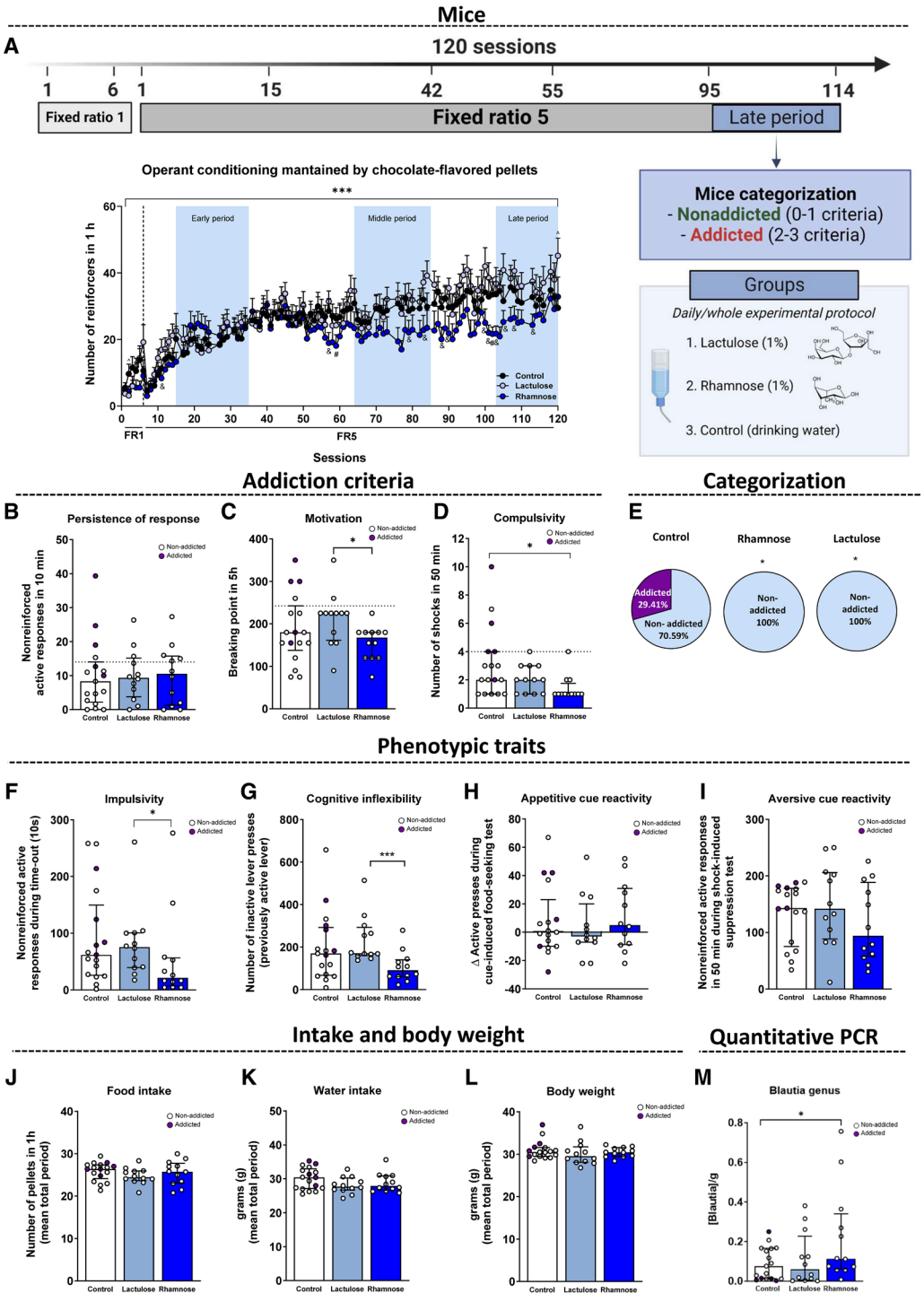

Figure 7 Characterisation of extreme subpopulations of addicted and non-addicted mice in the experiment with lactulose and rhamnose. (A) Timeline of the procedure of operant behaviour mouse model. Mice were trained during the first 6 days in operant behaviour sessions of 1 hour at a fixed ratio (FR) 1 schedule of reinforcement, followed by 114 daily sessions of FR5. The addiction-like criteria (persistence of response, motivation and compulsivity) were evaluated in the late period (95-114) to categorise mice into addicted and non-addicted. Mice received the non-digestible carbohydrate lactulose, rhamnose or control in drinking water during the whole experimental sequence. Number of reinforcers during 1 hour of operant training sessions maintained by chocolate flavoured pellets in the three groups (mean±SEM, repeated measures ANOVA, session × treatment effect ***p<0.001, post hoc Newman–Keuls, ^p<0.05 control vs lactulose, &p<0.05 lactulose vs rhamnose, #p<0.05 control vs rhamnose). (B–D) Behavioural tests for the three addiction-like criteria in the late period (individual values with IQR) in the addicted and non-addicted groups. (B) Persistence of response. (C) Motivation (Mann–Whitney U test, *p<0.05). (D) Compulsivity (Mann–Whitney U test, *p<0.05). (E) Percentage of mice classified as addicted and non-addicted in the lactulose, rhamnose and control groups. (F–I) Behavioural tests for the four phenotypic traits associated with vulnerability to food addiction in the late period (individual values with IQR). (F) Impulsivity. (G) Cognitive inflexibility (Mann–Whitney U test, ***p<0.001). (H) Appetitive cue reactivity. (I) Aversive cue reactivity. (J) Food intake. (K) Water intake. (L) Body weight. (M) (*Blautia*)/g in mice faeces determined by pPCR (Mann–Whitney U test, *p<0.05). The sample size of mice in the lactulose and rhamnose groups was n=12, and n=17 in the control group (total n=41). Statistical details are included in online supplemental table S3.

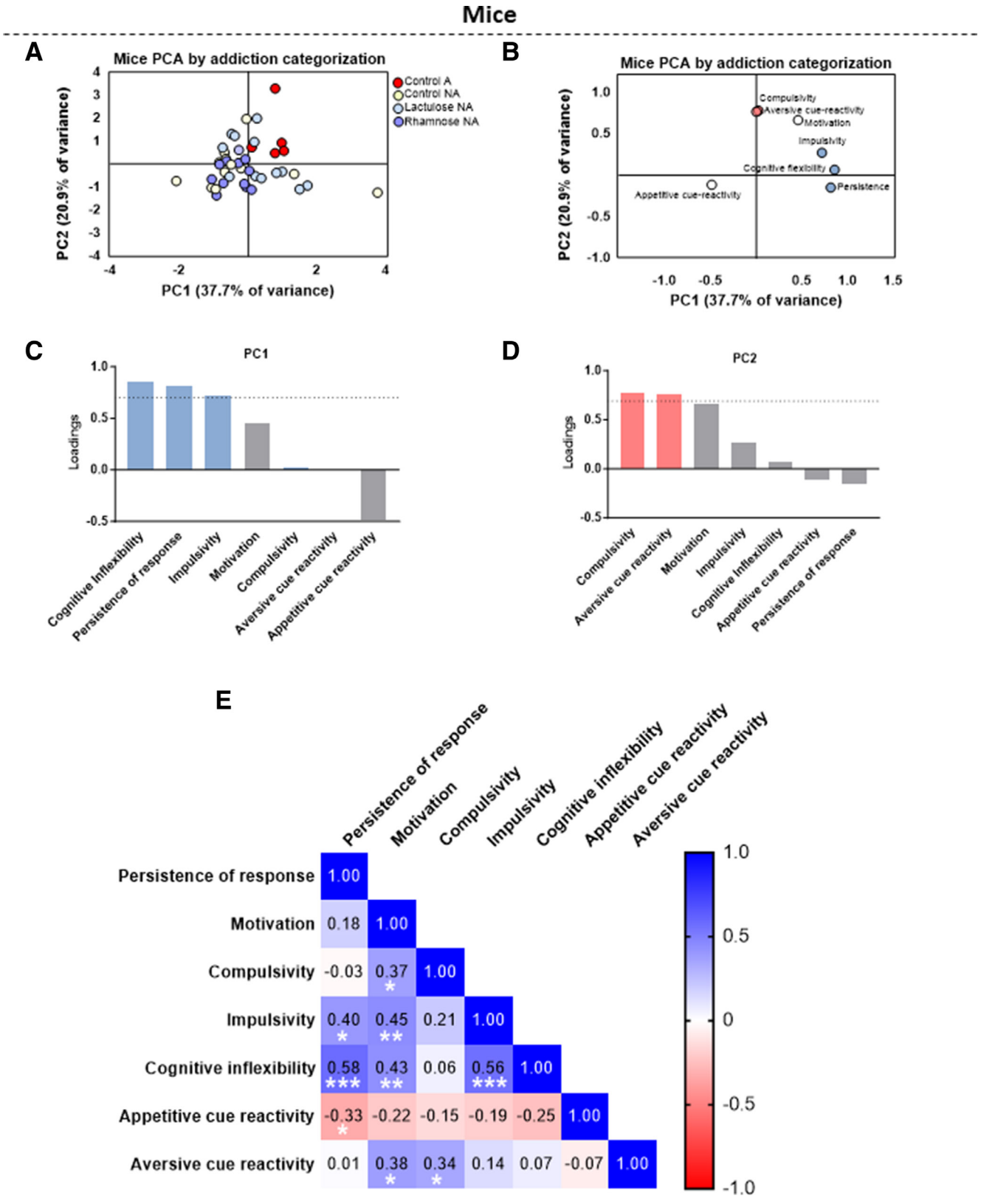

Figure 8 Principal component analysis (PCA) revealed differential patterns of behavioural factor loadings in food addiction-like behaviour in mice treated with lactulose and rhamnose. (A) Mice subjects clustered by addicted or non-addicted classification on the space yielded by two components of the PCA that account for the maximum data variance (n=5 control addicted mice, n=12 control non-addicted mice, n=12 lactulose non-addicted mice, n=12 rhamnose non-addicted mice). (B) Criteria belonging to each component, principal component (PC) 1 (37.7%) and PC2 (20.9%). (C, D) Order of factor loading of the different variables in PC1 and PC2 is represented. The dashed horizontal line marked loadings >0.7, mainly contributing to the component. (E) Heatmap correlation matrix of the three addiction criteria and the four phenotypic traits. Colours correspond to the magnitude of Pearson correlations between each pair of variables and range from –1 (red) to+1 (blue). Significant Pearson's correlations: *p<0.05, **p<0.01, ***p<0.001 (n=36 mice).

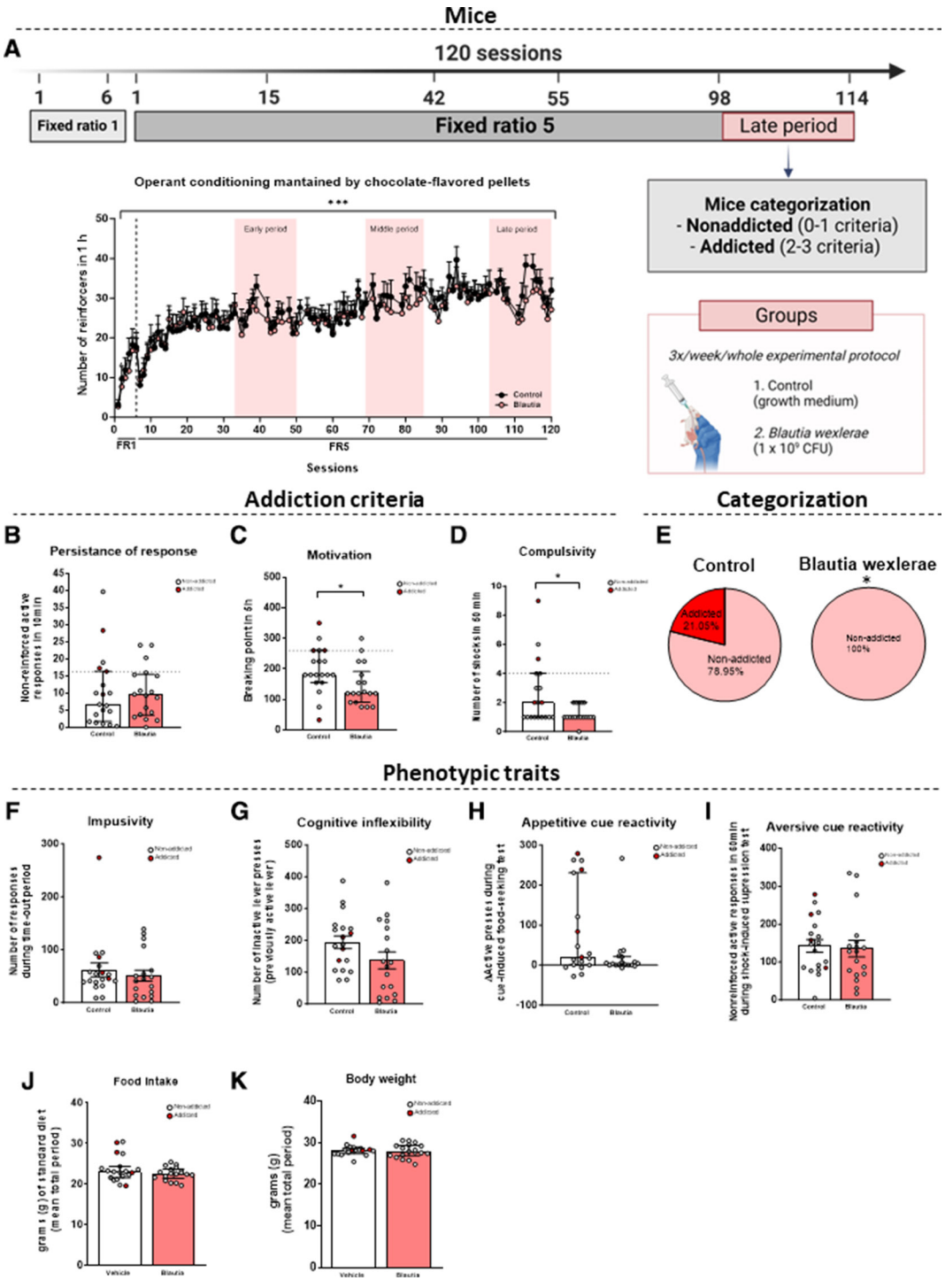

Figure 9 Characterisation of extreme subpopulations of addicted and non-addicted mice in the experiment with *Blautia wexlerae* supplementation. (A, upper part) Timeline of the procedure of operant behaviour mouse model. Mice were trained during the first 6 days in operant behaviour sessions of 1 hour at a fixed ratio (FR) 1 schedule of reinforcement, followed by 114 daily sessions of FR5. The addiction-like criteria (persistence of response, motivation and compulsivity) were evaluated in the late period (98-114) to categorise mice as addicted and non-addicted. Mice received the beneficial microbe *Blautia wexlerae* or vehicle control, which were administered by oral route (gavage) during the whole experimental sequence. Specifically, 250 µl of *Blautia wexlerae* were orally administered (intragavage) at a concentration of 1×10^9 CFU three times per week for the whole experimental protocol, 1 hour before the self-administration session in the operant chambers. (A bottom part) Number of reinforcers during 1 hour of operant training sessions maintained by chocolate flavoured pellets in the two groups (mean±SEM, repeated measures ANOVA, sessions, ***p<0.001). (B–D) Behavioural tests for the three addiction-like criteria in the late period (individual values with IQR) in the addicted and non-addicted groups. (B) Persistence of response. (C) Motivation (Mann–Whitney U test, *p<0.05). (D) Compulsivity (Mann–Whitney U test,*p<0.05). (E) Percentage of mice classified as addicted and non-addicted in the groups of *Blautia wexlerae* and control. (F–I) Behavioural tests for the four phenotypic traits associated with vulnerability to food addiction in the late period (individual values with IQR). (F) Impulsivity. (G) Cognitive inflexibility. (H) Appetitive cue reactivity. (I) Aversive cue reactivity. (J) Food intake. (K) Body weight. Sample size of mice in the *Blautia wexlerae* and vehicle control groups was n=18–19 per group (total n=37). Statistical details are included in online supplemental table S4.

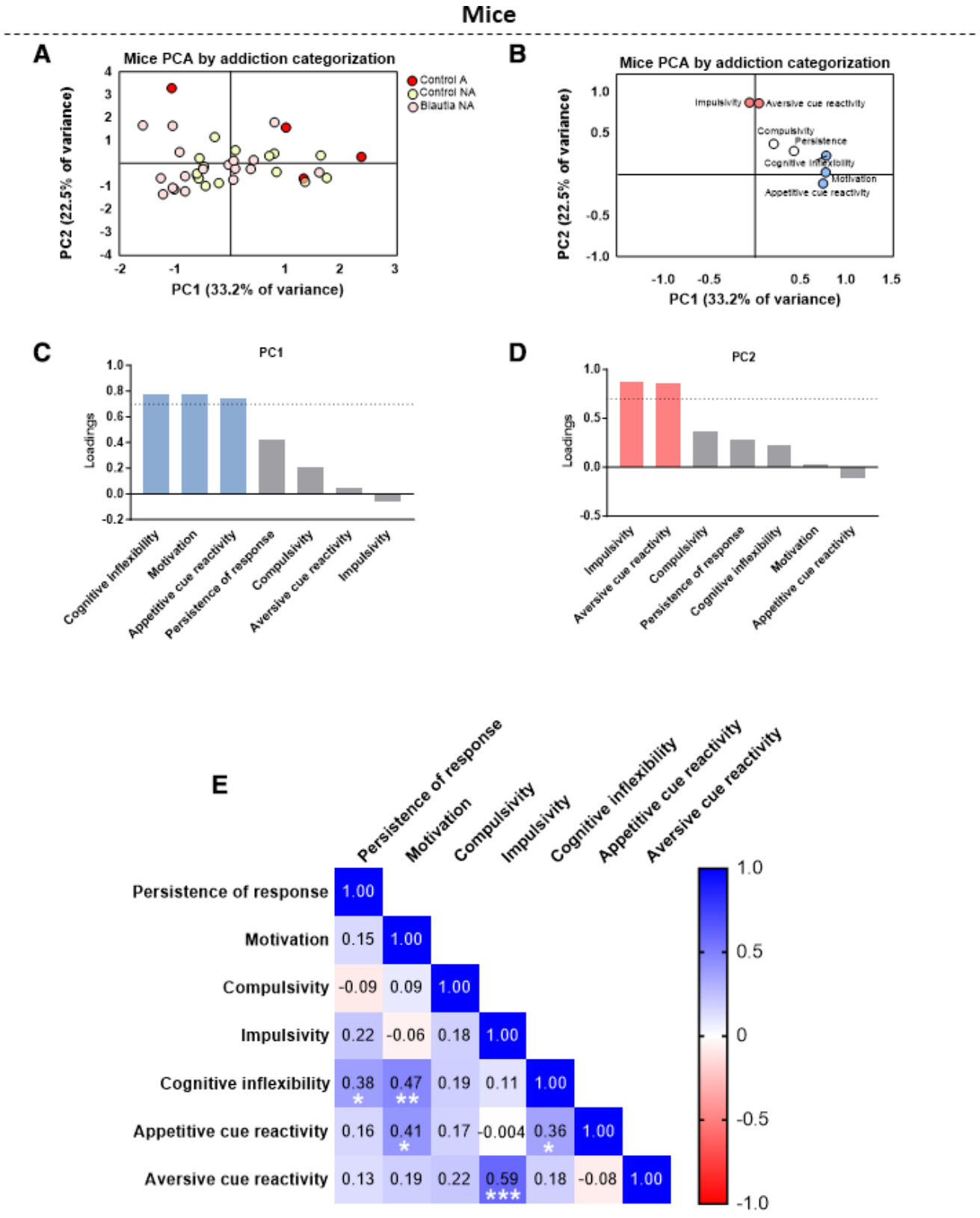

Figure 10 Principal component analysis (PCA) revealed differential patterns of behavioural factor loadings in food addiction-like behaviour in mice treated with *Blautia* wexlerae or control vehicle. (A) Mice subjects clustered by addicted or non-addicted classification on the space yielded by two components of the PCA that account for the maximum data variance (n=4 control addicted mice, n=15 control non-addicted mice, n=18 *Blautia wexlerea* non-addicted mice). (B) Criteria belonging to each component, principal component (PC) 1 (33.2%) and PC2 (22.5%). (C, D) Order of factor loading of the different variables in PC1 and PC2 is represented. The dashed horizontal line marked loadings >0.7, mainly contributing to the component. (E) Heatmap correlation matrix of the three addiction criteria and the four phenotypic traits. Colours correspond to the magnitude of Pearson correlations between each pair of variables and range from −1 (red) to +1 (blue). Significant Pearson's correlations: *p<0.05, **p<0.01, ***p<0.001 (n=37 mice).

non-beneficial or beneficial effects, depending on each species.[32] Another study exploring alcohol use disorder vulnerability in rats also showed opposite results in different genera of *Lachnospiraceae* family, with opposite correlations between dorsal striatum dopamine D2 receptor (D2R) expression and relative abundances, also suggesting non-beneficial or beneficial effects.[6]

The *Allobaculum* genus, which also belongs to the Bacillota/Firmicutes phylum, was potentially protective in our mouse food addiction model. Chronic alcohol exposure was reported to increase *Allobaculum* spp. abundance in mice positively related to alcohol preference, which suggests a non-beneficial effect of this genus.[33] In contrast, beneficial effects of the *Allobaculum* genus were reported in an obesity mouse model with the anti-obesity diet quercetin.[34] Furthermore, *Allobaculum* positively correlated with dorsal striatum D2R expression in a rat model of alcohol addiction, suggesting a beneficial effect,[6] in agreement with the potential protective effect of this bacteria in our food addiction model. Importantly, modifications in *drd2* gene expression and other dopaminergic markers of the reward circuit have been reported in our mouse model of food addiction.[13]

Blautia genus, belonging to the *Lachnospiraceae* family, was downregulated in addicted mice and is an essential player in the microbiota gut–brain axis. Striking similarities were observed in *Blautia* content in mice and humans vulnerable to developing food addiction, and these bacteria could have a relevant potential beneficial role in the regulation of brain function,[26 35] as discussed and validated later.

Our results in mice also suggested non-beneficial effects on several bacteria groups, such as the *Anaeroplasma* genus and *Gastranaerophilales* family. The relative abundance of *Anaeroplasma* genus (from Tenericutes phylum and *Anaeroplasmataceae* family) was increased in addicted mice. An earlier study demonstrated the antiobesity activity of thinned peach polyphenols, which had beneficial effects on gut microbiota by reducing the *Anaeroplasma* abundance that positively correlated with obesity.[36] In agreement with the potential negative effects of *Gastranaerophilales*, the beneficial activity of hawthorn seed oil supplementation was reported to be related to the reduction of this family.[36] In our study, the *Gastranaerophilales* family was specifically associated with the addiction-like criterion of motivation.

The *Clostridiales_vandin BB60* and *Ruminococcaceae* families, both from the Bacillota/Firmicutes phylum, positively correlated with the addiction criterion of persistence of response in addicted mice and positively correlated between them, suggesting a joint action of these clusters of bacteria. Alterations of Ruminococcaceae or Clostridiales bacterial abundances have been reported in patients with autism spectrum disorders, schizophrenia and social behavioural disorder,[37 38] which further supports the possibility that the gut–brain axis may affect the persistence of response. In agreement, an increased abundance of *Clostridiales vadinBB660* has been reported in the maternal separation model in rats,[39] associating these bacteria with responsiveness to stress.[40] Furthermore, trends of increased *Ruminococcaceae* and *Clostridiales vadinBB660* families were revealed in vulnerable rats to develop alcohol use disorder.[6] All of these findings together suggest that clusters of bacteria are addiction responsive bacteria that may potentially affect host behaviours.

Our animal and human cohorts identified important similarities in the behavioural characterisation of food addiction and the gut microbiota signatures associated with this behaviour. Microbiota is a regulator of the reward system,[6] and gut microbiota derived metabolites are critical regulators of host appetite.[41] In our human cohort, we observed significant differences in the relative abundances of bacteria belonging to Actinobacteria, Bacillota/Firmicutes and Proteobacteria phyla that were in the same direction to what was found in the mice cohort, demonstrating the high translational relevance of the results obtained in animals and humans. Notably, *Blautia species* were downregulated in addicted individuals, which could be linked to beneficial effects in parallel with what was found in mice at the genus level. Furthermore, the species *Lactobacillus kefiri* (from the *Lactobacillaceae* family and Bacillota/Firmicutes phylum) was also reduced in humans diagnosed as food addicted, suggesting a potential beneficial effect, similar to what was described for mice at the family level. Although no effects have been reported regarding the influence of this bacteria on food addiction, previous studies have reported the beneficial effects of dairy intake of *Lactobacillus kefiri*.[42 43] Concerning the Proteobacteria phylum, an increased relative abundance of several species was found in individuals in our human cohort with a high score in food addiction, in coherence with the results in mice that suggested a non-beneficial effect of this phylum. In previous studies, Proteobacteria was observed to be differentially abundant among individuals diagnosed with schizophrenia, with patients having an increased relative abundance of this phylum compared with the healthy cohort.[44] *Proteobacteria* species are considered proinflammatory gut bacteria, and patients with alcoholism and dysbiosis had higher abundances of Proteobacteria than subjects without alcoholism.[45]

From all of these similarities between mouse and human gut microbiota associated with food addiction vulnerability, it is important to underline the findings revealed on *Blautia wexlerae* species and *Blautia* genus. *Blautia wexlerae*, *Blautia schinkii and Blautia gluceraseasa* species were upregulated in non-addicted human individuals, consistent with the increase of the *Blautia* genus in non-addicted mice. These results suggest a beneficial effect of these bacteria on food addiction. Previous studies have identified other bacteria associated with food addiction, precisely a protective effect of Bacteroides, Akkermansia and Eubacterium genus and a risky association with megamonas in human females.[46] Other studies have observed that gut microbiota reduction in mice increases binge-like eating of palatable food.[47] In obese women with uncontrolled eating behaviour, another study described peculiar gut microbiome clusters associated with different eating patterns,[19] which underlies the association of different endophenotypes with distinct microbiome signatures. Importantly, the microbiota has been associated with dysregulations of the dopaminergic reward system and the hedonic food intake during obesity.[16]

To date, 20 species constitute the genus *Blautia,* including *Blautia coccoides*, *Blautia wexlerae*, *Blautia schinkii*, *Blautia gluceraseasa and Blautia producta*.[48] Both *Blautia* genus and *Lachnospiraceae* family that includes this genus belong to the important Bacillota/Firmicutes phylum. *Lachnospiraceae* is the most abundant family of this phylum, accounting for approximately 50% of the total gut microbiota in humans.[49] *Blautia* genus is involved in host bile transformation[28] that activates serotonin synthesis. Previous studies with methamphetamine consumption in humans showed contradictory results with increased *Blautia* proportions in abusers, suggesting non-beneficial effects, but also a positive association with abstraction in a cognitive assessment.[50] Other studies reported beneficial effects on obesity. Thus breastfeeding is associated with a reduced risk of obesity and comorbidities later in life, and a microbiota profile driven by the genus *Blautia* was linked to beneficial metabolic effects.[35] *Blautia* has also received particular attention for its involvement in improving metabolic diseases and promoting nutritional

advantages.[51] In addition, *Blautia* has shown potential properties considering its ability to regulate host health and improve metabolic syndrome, inhibiting the colonisation of pathogenic bacteria in the intestine.[48] However, its production requires restrictive culture conditions due to its nature of strictly anaerobic bacteria, which makes it difficult for a potential use as a beneficial microbe for human therapeutic purposes. Given the role of *Blautia* in metabolic host regulation, using non-digestible carbohydrates as substrates to promote *Blautia* proliferation may represent a promising alternative to potentiate its beneficial functions. Non-digestible carbohydrates, such as lactulose and rhamnose, are promising candidates that have been shown to increase the abundance of *Blautia* and cause beneficial effects on the host.[24 25 52] In the current study, we functionally validated the potential protective effects of *Blautia* in the development of food addiction by administering non-digestible carbohydrates, and showing increased relative abundance of *Blautia* in mice faeces in the case of rhamnose. Both non-digestible carbohydrates had a protective effect since their administration completely avoided the development of addictive-like behaviour in mice. This beneficial impact was more pronounced in the case of rhamnose, with more significant outcomes than lactulose. Furthermore, we validated the specific involvement of *Blautia* in these responses by orally administering *Blautia wexlerae* in mice as a beneficial microbe. Oral *Blautia* administration also has a similar protective effect, avoiding the development of food addiction in mice with a predominant effect in preventing motivation and compulsivity. In agreement, oral administration of *Blautia wexlerae* was reported to ameliorate obesity and type 2 diabetes via metabolic remodelling of the gut microbiota.[26] The live biotherapeutic strain, *Blautia stercoris* MRx0006, was recently demonstrated to have beneficial behavioural effects in a mouse model of autism.[53]

Our study identified *Blautia* as a target of potential interest for food addiction treatment and prevention. In agreement, a recent study showed the association between *Blautia* and cocaine reinforcing properties in an intravenous cocaine self-administration model in mice, although the relevance of this association was not investigated, and machine learning approaches also suggest a similar association with substance use disorder in humans.[54 55] We also evaluated gene expression signatures related to food addiction targeting the dopaminergic system in the mPFC and NAc after *Blautia* administration, considering the alterations in dopaminergic markers that we previously revealed in food addicted mice.[13] No significant differences in gene expression were obtained between mice treated with *Blautia* or vehicle when evaluating four main targets of the dopaminergic system by qPCR in these two brain areas: *Drd2* (dopamine receptor type 2), *Drd1* (dopamine receptor type 1), *Th* (tyrosine hydroxylase), and *Darpp32* (dopamine and cAMP regulated neuronal phosphoprotein). These results suggest that the beneficial effects of *Blautia* in the behavioural expression of food addiction were not associated with major changes in these four markers of dopamine activity in the mPFC and NAc. Multiple other neurochemical mechanisms and pathways are involved in the loss of eating control[56–58] and the influence of the gut–brain axis in this behaviour,[59] opening new possibilities of research to elucidate the precise mechanisms participating in the involvement of gut microbiota in the loss of eating control.

We demonstrated a translational link between mice and humans in gut microbiome composition associated with food addiction, supporting a link between gut microbiota and vulnerability to this behavioural disorder. The gut microbiome composition found in our study was also associated with specific addiction criteria, including motivation and persistence of response, in a food addiction mouse model based on the human YFAS 2.0 questionnaire with marked similarities with findings in our human cohort, substantiating the translational value of the model. The functional relevance and beneficial effects of *Blautia*, the most relevant similar findings revealed in mice and human gut microbiota, was demonstrated by orally administering *Blautia wexlerae* in mice, as well as by administering lactulose and rhamnose, non-digestible carbohydrates that increased the relative abundance of *Blautia* in mice faeces and that also prevented the development of food addiction. These observations underlie the possible role of the gut microbiome in predisposing individuals to food addiction and offer a perspective on understanding the aetiology of food addiction that remains essentially unknown. Identification of non-beneficial bacteria can be helpful as prognostic biomarkers for predicting vulnerability to food addiction that may lead to a real improvement in clinical care. This novel understanding of the role of gut microbiota in the development of food addiction may open new approaches for developing biomarkers and innovative therapies for food addiction and related eating disorders.

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Contributors EM-G and RM conceived and designed the experimental approaches in animal studies. JMF-R conceived and designed the experimental approaches in humans. AB and LD-R performed the behavioral phenotype characterisation of the first batch of mice with the supervision of EM-G and RM. AG-B, SS, JC-D, LP-C, NF-C and LD-R performed statistical analyses and graphs with the supervision of EM-G and RM. SA performed the DNA extractions, DNA library preparation for sequencing and analyses supervised by CS. AB and SA made mice microbiota data analysis and graphs. JL and JM-P performed the studies in humans with the supervision of JMF-R. JE-C performed the bioinformatic analysis. SS performed the behavioral experiment with non-digestible carbohydrates and qPCR for Blautia abundance with the supervision of EM-G and RM. SS performed the experiment with the Blautia beneficial microbe with the collaboration of AG-B and supervision of EM-G and RM. JK and KH provided the Blautia beneficial microbe for the food addiction experiment in mice. EG-G performed the qPCR experiment for gene expression in the mouse brain with the supervision of NF-C and BC. EM-G and RM wrote the manuscript,

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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REFERENCES

1 Li M, Wang B, Zhang M, *et al*. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A* 2008;105:2117–22.
2 Mayneris-Perxachs J, Castells-Nobau A, Arnoriaga-Rodríguez M, *et al*. Microbiota alterations in proline metabolism impact depression. *Cell Metab* 2022;34:681–701.
3 Sen P, Sherwin E, Sandhu K, *et al*. The live biotherapeutic blautia stercoris MRx0006 attenuates social deficits, repetitive behaviour, and anxiety-like behaviour in a mouse model relevant to autism. *Brain Behav Immun* 2022;106:115–26.
4 Shen S, Zhao J, Dai Y, *et al*. Methamphetamine-induced alterations in intestinal mucosal barrier function occur via the microRNA-181c/ TNF-α/tight junction axis. *Toxicol Lett* 2020;321:73–82.
5 Leclercq S, Matamoros S, Cani PD, *et al*. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci U S A* 2014;111:E4485–93.
6 Jadhav KS, Peterson VL, Halfon O, *et al*. Gut microbiome correlates with altered striatal dopamine receptor expression in a model of compulsive alcohol seeking. *Neuropharmacology* 2018;141:249–59.
7 Meckel KR, Kiraly DD. A potential role for the gut microbiome in substance use disorders. *Psychopharmacology (Berl)* 2019;236:1513–30.
8 Gupta A, Osadchiy V, Mayer EA. Brain–gut–microbiome interactions in obesity and food addiction. *Nat Rev Gastroenterol Hepatol* 2020;17:655–72.
9 Pursey KM, Stanwell P, Gearhardt AN, *et al*. The prevalence of food addiction as assessed by the Yale food addiction scale: a systematic review. *Nutrients* 2014;6:4552–90.
10 Randolph TG. The descriptive features of food addiction: addictive eating and drinking. *Q J Stud Alcohol* 1956;17:198–224.
11 American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, 5th edition (DSM-5)*. Washington, DC. USA, 2013.
12 Gearhardt AN, Corbin WR, Brownell KD. Development of the Yale food addiction scale version 2.0. *Psychol Addict Behav* 2016;30:113–21.
13 Domingo-Rodriguez L, Ruiz de Azua I, Dominguez E, *et al*. A specific prelimbic-nucleus accumbens pathway controls resilience versus vulnerability to food addiction. *Nat Commun* 2020;11:782.
14 García-Blanco A, Domingo-Rodriguez L, Cabana-Domínguez J, *et al*. miRNA signatures associated with vulnerability to food addiction in mice and humans. *J Clin Invest* 2022;132:e156281.
15 Mancino S, Burokas A, Gutiérrez-Cuesta J, *et al*. Epigenetic and proteomic expression changes promoted by eating addictive-like behavior. *Neuropsychopharmacology* 2015;40:2788–800.
16 de Wouters d'Oplinter A, Rastelli M, Van Hul M, *et al*. Gut microbes participate in food preference alterations during obesity. *Gut Microbes* 2021;13.
17 Agustí A, Campillo I, Balzano T, *et al*. Bacteroides uniformis CECT 7771 modulates the brain reward response to reduce binge eating and anxiety-like behavior in rat. *Mol Neurobiol* 2021;58:4959–79.
18 Huwart SJP, de Wouters d'Oplinter A, Rastelli M, *et al*. Food reward alterations during obesity are associated with inflammation in the striatum in mice: beneficial effects of akkermansia muciniphila. *Cells* 2022;11:2534.
19 Barone M, Garelli S, Rampelli S, *et al*. Multi-omics gut microbiome signatures in obese women: role of diet and uncontrolled eating behavior. *BMC Med* 2022;20:500.
20 Narmaki E, Borazjani M, Ataie-Jafari A, *et al*. The combined effects of probiotics and restricted calorie diet on the anthropometric indices, eating behavior, and hormone levels of obese women with food addiction: a randomized clinical trial. *Nutr Neurosci* 2022;25:963–75.
21 Arnoriaga-Rodríguez M, Mayneris-Perxachs J, Burokas A, *et al*. Obesity impairs short-term and working memory through gut microbial metabolism of aromatic amino acids. *Cell Metab* 2020;32:548–60.
22 Salminen S, Collado MC, Endo A, *et al*. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol* 2021;18:649–67.
23 Megur A, Daliri EB-M, Baltriukienė D, *et al*. Prebiotics as a tool for the prevention and treatment of obesity and diabetes: classification and ability to modulate the gut microbiota. *Int J Mol Sci* 2022;23:6097.
24 Cui S, Gu J, Liu X, *et al*. Lactulose significantly increased the relative abundance of bifidobacterium and blautia in mice feces as revealed by 16S rRNA amplicon sequencing. *J Sci Food Agric* 2021;101:5721–9.
25 Reichardt N, Vollmer M, Holtrop G, *et al*. Specific substrate-driven changes in human faecal microbiota composition contrast with functional redundancy in short-chain fatty acid production. *ISME J* 2018;12:610–22.

26 Hosomi K, Saito M, Park J, *et al*. Oral administration of Blautia wexlerae ameliorates obesity and type 2 diabetes via metabolic remodeling of the gut microbiota. *Nat Commun* 2022;13:4477.
27 Claesson MJ, Jeffery IB, Conde S, *et al*. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012;488:178–84.
28 Golubeva AV, Joyce SA, Moloney G, *et al*. Microbiota-related changes in bile acid & tryptophan metabolism are associated with gastrointestinal dysfunction in a mouse model of autism. *EBioMedicine* 2017;24:166–78.
29 Moloney GM, Dinan TG, Clarke G, *et al*. Microbial regulation of microRNA expression in the brain–gut axis. *Curr Opin Pharmacol* 2019;48:120–6.
30 Deng Y, Zhou M, Wang J, *et al*. Involvement of the microbiota-gut-brain axis in chronic restraint stress: disturbances of the kynurenine metabolic pathway in both the gut and brain. *Gut Microbes* 2021;13:1–16.
31 Kieser S, Zdobnov EM, Trajkovski M. Comprehensive mouse microbiota genome catalog reveals major difference to its human counterpart. *PLoS Comput Biol* 2022;18:e1009947.
32 Wang G, Liu Q, Guo L, *et al*. Gut microbiota and relevant metabolites analysis in alcohol dependent mice. *Front Microbiol* 2018;9:1874.
33 Xu Z, Wang C, Dong X, *et al*. Chronic alcohol exposure induced gut microbiota dysbiosis and its correlations with neuropsychic behaviors and brain BDNF/Gabra1 changes in mice. *Biofactors* 2019;45:187–99.
34 Su L, Zeng Y, Li G, *et al*. Quercetin improves high-fat diet-induced obesity by modulating gut microbiota and metabolites in C57BL / 6J mice. *Phytother Res* 2022;36:4558–72.
35 Freitas R de O, Vasques ACJ, Fernandes G da R, *et al*. Associations of Blautia genus with early-life events and later phenotype in the nutrihs. *Front Cell Infect Microbiol* 2022;12:838750.
36 Dai K, Song Y, Zhang D, *et al*. Thinned peach polyphenols alleviate obesity in high fat mice by affecting gut microbiota. *Food Res Int* 2022;157:111255.
37 Adams JB, Johansen LJ, Powell LD, *et al*. Gastrointestinal flora and gastrointestinal status in children with autism - comparisons to typical children and correlation with autism severity. *BMC Gastroenterol* 2011;11:22.
38 Nguyen TT, Kosciolek T, Maldonado Y, *et al*. Differences in gut microbiome composition between persons with chronic schizophrenia and healthy comparison subjects. *Schizophr Res* 2019;204:23–9.
39 Rincel M, Olier M, Minni A, *et al*. Pharmacological restoration of gut barrier function in stressed neonates partially reverses long-term alterations associated with maternal separation. *Psychopharmacology (Berl)* 2019;236:1583–96.
40 Usui N, Matsuzaki H, Shimada S. Characterization of early life stress-affected gut microbiota. *Brain Sci* 2021;11:1–10.
41 Han H, Yi B, Zhong R, *et al*. From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. *Microbiome* 2021;9:162.
42 Kim D-H, Jeong D, Kang I-B, *et al*. Dual function of Lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: direct reduction of cholesterol and upregulation of PPAR-A in adipose tissue. *Mol Nutr Food Res* 2017;61.
43 Zubiría MG, Gambaro SE, Rey MA, *et al*. Deleterious metabolic effects of high fructose intake: the preventive effect of lactobacillus kefiri administration. *Nutrients* 2017;9:470.
44 Shen Y, Xu J, Li Z, *et al*. Analysis of gut microbiota diversity and auxiliary diagnosis as a biomarker in patients with schizophrenia: a cross-sectional study. *Schizophr Res* 2018;197:470–7.
45 Mutlu EA, Gillevet PM, Rangwala H, *et al*. Colonic microbiome is altered in alcoholism. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G966–78.
46 Dong TS, Mayer EA, Osadchiy V, *et al*. A distinct brain-gut-microbiome profile exists for females with obesity and food addiction. *Obesity (Silver Spring)* 2020;28:1477–86.
47 Ousey J, Boktor JC, Mazmanian SK. Gut microbiota suppress feeding induced by palatable foods. *Curr Biol* 2023;33:147–57.
48 Liu X, Mao B, Gu J, *et al*. Blautia—a new functional genus with potential probiotic properties? *Gut Microbes* 2021;13:1–21.
49 Biddle A, Stewart L, Blanchard J, *et al*. Untangling the genetic basis of fibrolytic specialization by lachnospiraceae and ruminococcaceae in diverse gut communities. *Diversity* 2013;5:627–40.
50 Yang C, Fu X, Hao W, *et al*. Gut dysbiosis associated with the rats' responses in methamphetamine-induced conditioned place preference. *Addict Biol* 2021;26:e12975.
51 Khattab MSA, Tawab A, Fouad MT. Isolation and characterization of anaerobic bacteria from frozen rumen liquid and its potential characterizations. *Int J Dairy Sci* 2016;12:47–51.
52 Lv S, Hu T, Zhang R, *et al*. Rhamnose displays an anti-obesity effect through stimulating adipose dopamine receptors and thermogenesis. *Diabetes* 2023;72:326–35.
53 Sen P, Sherwin E, Sandhu K, *et al*. The live biotherapeutic blautia stercoris MRx0006 attenuates social deficits, repetitive behaviour, and anxiety-like behaviour in a mouse model relevant to Autism. *Brain Behav Immun* 2022;106:115–26.
54 Liu Y, Zhang P, Sheng H, *et al*. 16S rRNA gene sequencing and machine learning reveal correlation between drug abuse and human host gut microbiota. *Addict Biol* 2023;28:1–10.
55 Binh Tran TD, Nguyen H, Sodergren E, *et al*. Microbial glutamate metabolism predicts intravenous cocaine self-administration in diversity outbred mice. *Neuropharmacology* 2023;226:109409.
56 Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry* 2016;3:760–73.
57 Volkow ND, Wang G-J, Tomasi D, *et al*. Obesity and addiction: neurobiological overlaps. *Obes Rev* 2013;14:2–18.
58 Maldonado R, Calvé P, García-Blanco A, *et al*. Vulnerability to addiction. *Neuropharmacology* 2021;186:108466.
59 Novelle MG. Decoding the role of gut-microbiome in the food addiction paradigm. *Int J Environ Res Public Health* 2021;18:6825.