BRAIN
ORIGINAL ARTICLE

Microbiota from Alzheimer's patients induce deficits in cognition and hippocampal neurogenesis

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Alzheimer's disease is a complex neurodegenerative disorder leading to a decline in cognitive function and mental health. Recent research has positioned the gut microbiota as an important susceptibility factor in Alzheimer's disease by showing specific alterations in the gut microbiome composition of Alzheimer's patients and in rodent models. However, it is unknown whether gut microbiota alterations are causal in the manifestation of Alzheimer's symptoms.

To understand the involvement of Alzheimer's patient gut microbiota in host physiology and behaviour, we transplanted faecal microbiota from Alzheimer's patients and age-matched healthy controls into microbiota-depleted young adult rats. We found impairments in behaviours reliant on adult hippocampal neurogenesis, an essential process for certain memory functions and mood, resulting from Alzheimer's patient transplants. Notably, the severity of impairments correlated with clinical cognitive scores in donor patients. Discrete changes in the rat caecal and hippocampal metabolome were also evident. As hippocampal neurogenesis cannot be measured in living humans but is modulated by the circulatory systemic environment, we assessed the impact of the Alzheimer's systemic environment on proxy neurogenesis readouts. Serum from Alzheimer's patients decreased neurogenesis in human cells *in vitro* and were associated with cognitive scores and key microbial genera.

Our findings reveal for the first time, that Alzheimer's symptoms can be transferred to a healthy young organism via the gut microbiota, confirming a causal role of gut microbiota in Alzheimer's disease, and highlight hippocampal neurogenesis as a converging central cellular process regulating systemic circulatory and gut-mediated factors in Alzheimer's.

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Introduction

Alzheimer's disease, the leading cause of dementia, is a complex neurodegenerative disorder characterized by progressive impairments in cognitive function, which are often comorbid with neuropsychiatric symptoms.[1-3] Neuropathological hallmarks of Alzheimer's disease include extracellular deposition of amyloid-β (Aβ) plaques, intraneuronal accumulation of neurofibrillary tangles composed of hyperphosphorylated tau, neuroinflammation and neuronal death.[4-6] The hippocampus plays a critical role in learning and memory[7] and is particularly vulnerable to Alzheimer's pathology, being one of the earliest brain areas to be affected.[8] The hippocampus is also host to a population of neural stem cells, which generate new neurons throughout the lifespan in a process called adult hippocampal neurogenesis (AHN).[9,10] This unique form of cellular plasticity is a key mediator of cognitive functions such as spatial learning, pattern separation (an ability to distinguish between highly similar events or environments),[11-13] as well as the regulation of emotion.[14] Notably, these behaviours are impaired in Alzheimer's disease.[15-17] Crucially, AHN is altered in early Braak stages, preceding neurofibrillary tangles and Aβ plaque formation in the hippocampus of humans,[18] suggesting that AHN dysfunction is an early feature of Alzheimer's pathogenesis.[19]

It is increasingly recognized that Alzheimer's is a multifactorial disease, substantially influenced by genetic, lifestyle and environmental factors.[20-22] While it is well established that changes in the systemic circulatory environment may accelerate the development of Alzheimer's pathology,[23] the gut microbiome is now emerging as a key target for investigation in Alzheimer's disease due to its particular susceptibility to lifestyle and environmental influences.[24-26] Moreover, the microbiota–gut–brain axis is now recognized as a significant mediator of behaviour throughout the lifespan.[27,28] Altered microbial composition, microbial metabolites and bacterial endotoxins have been reported in Alzheimer's patients.[29-36] Recently, a significant genetic overlap and correlation between Alzheimer's disease and gastrointestinal tract disorders has been suggested.[37]

Faecal microbiota transplantation (FMT) studies using mouse models of Alzheimer's disease have implicated the gut microbiota in pathological features of Alzheimer's disease; endoplasmic reticulum stress in recipient mice is enhanced after FMT from APP/PS1 mice and Alzheimer's patients,[38] and transplantation of gut microbiota derived from a 5xFAD mouse model of Alzheimer's disease impairs spatial memory and AHN in C57BL/6 mice.[39] Notably, the microbiota–gut–brain axis has been shown to regulate AHN.[40-42] However, it is as yet unexplored if cognitive symptoms in human Alzheimer's patients coupled with underlying cellular changes such as AHN can be transmitted to a healthy organism via the gut microbiota to thus position the gut microbiota as a critical mediator of Alzheimer's symptomology.

We addressed this using FMT from Alzheimer's participants to young adult rats to determine behavioural, neurogenic and metabolic features mediated through gut microbiota. The influence of the systemic milieu of Alzheimer's participants on hippocampal neurogenesis was assessed in parallel in human hippocampal progenitor cells *in vitro* to establish AHN as a converging central cellular process from systemic circulatory and gut-mediated factors in Alzheimer's disease.

Materials and methods

Human participants

Patients with Alzheimer's disease ($n = 69$) and cognitively healthy control subjects ($n = 64$) were recruited at the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. All participants underwent clinical assessment for cognitive function and a physical examination. See Supplementary material for details.

Human biological samples

Venous blood was sampled from an antecubital vein between 8–10 a.m. and collected in EDTA tubes (BD 186 Vacutainer Systems) for preparation of plasma. Stool samples were collected from a subset of participants ($n = 54$ for Alzheimer's disease and $n = 41$ for controls) at their own home in a sterile plastic cup, stored at −20°C and subsequently delivered to IRCCS Centro San Giovanni di Dio Fatebenefratelli, for storage at −20°C until processing. For stool used in FMT to rats, frozen samples were transferred into an anaerobic chamber, thawed and homogenized at a 100 mg/ml concentration in reduced sterile PBS containing 20% glycerol (w/v) and filtered through a stomacher bag. The filtered slurry was aliquoted and frozen at −80°C. Samples were defrosted immediately before inoculating recipient rats.

Human immune profile

For cytokine expression analysis in plasma, total RNA was isolated using the PAXgene blood miRNA kit according to the manufacturer's protocol (PreAnalytiX) and gene expression analyses was performed using real-time PCR (Supplementary material).

Animals

To avoid effects of ageing, adult male Sprague-Dawley rats (310–350 g) aged 11 weeks, (Envigo) were used and maintained in environmentally controlled conditions at the ambient temperature of 21°C ± 2°C, 55 ± 10% humidity, and under a 12-h light-dark cycle. Animals were group-housed (four per cage) in autoclaved cages with autoclaved bedding (Eco-Pure Aspen chips) and had access to autoclaved water and chow (Teklad Global Diet 2018S; Envigo) *ad libitum*.

Faecal microbiota transplantation

Following 2 weeks acclimatization, rats were administered an antibiotic cocktail of ampicillin (1 g/l), vancomycin (500 mg/l), ciprofloxacin HCL (200 mg/l) and imipenem (250 mg/l) for seven consecutive days in autoclaved drinking water, which has

previously been shown to ensure sufficient microbiota depletion.[43,44] Rats were colonized 72 h later via daily oral gavage of donor microbiota (300 µl of 100 mg/ml homogenized faecal slurry) for 3 days.[45] See Supplementary material for details.

Study design and experimental timeline for animal experiments

After 7 days of antibiotics, rats were randomly allocated into one of the two groups, balanced by weight (using a random number table): rats receiving FMT from control subjects (control FMT, $n = 16$) and rats receiving FMT from Alzheimer's patients (AD-FMT, $n = 16$). See Supplementary material for details.

Faecal sample collection and faecal water content in rats

Faecal samples were collected and faecal water content determined as previously described.[46] (Supplementary material).

16S microbiota and bioinformatic analysis of human and rat samples

DNA was extracted from 180–200 mg of frozen stool using the QIAamp DNA Stool Mini Kit (Qiagen Retsch GmbH) according to the manufacturer's instructions. For details and bioinformatic analysis see Supplementary material.

Histological analysis of ileal and colonic sections from rats

Colon and ileum sections were prepared using the Swiss-roll technique. After fixation, Swiss-rolls were embedded in paraffin and stained with haematoxylin and eosin. Alcian Blue/Periodic acid-Schiff (PAS) staining was performed to identify goblet cells (Supplementary material).

Behavioural testing in rats

Behavioural testing started 10 days after the first inoculation and behaviours were conducted between 9 a.m. and 7 p.m. during the light phase of the light cycle with a minimum of 36 h between each behavioural test. Rats underwent a battery of behavioural tests conducted in the following order: Open Field, Elevated Plus Maze, Modified Spontaneous Location Recognition Test, Novel Object Recognition, Novel Location Recognition, Morris Water Maze and Forced Swim Test. A detailed description of the behavioural tests can be found in the Supplementary material.

Immunohistochemistry of rat brain tissue

BrdU/NeuN, DCX, Ki67 and Iba-1 immunohistochemistry was performed in the hippocampus, as previously described.[47,48] See Supplementary material for details of immunohistochemistry, analysis of cell density and 3D reconstruction of DCX + cells.

Thioflavin S staining

For visualization of amyloid plaques, Thioflavin S staining was carried out on hippocampal and cortex sections (Supplementary material).

Cell culture and serum treatment

All experiments were performed using the multipotent human hippocampal progenitor/stem cell line HPC0A07/03C (HPC, ReNeuron) derived from the first trimester female foetal hippocampal tissue following medical termination (in accordance with the UK and USA ethical and legal guidelines, and obtained from Advanced Bioscience Resources). See Supplementary material for details on cell culture, serum treatments, immunocytochemistry, high-content imaging and neurite outgrowth analysis.

Rat hippocampal and caecal metabolomics

Metabolomic analysis was carried out on caecum and hippocampus by Ms-OMICS. See Supplementary material for details on the preparation of hippocampal tissue and caecal content, mass spectrometry and bioinformatic analysis.

Quantitative real-time PCR for immune profile in rat colon

Isolation of total RNA was performed using the GenElute kit (Sigma) as described by the manufacturer (Supplementary material).

Cytokine assay in rat plasma

Tail blood (10 days after FMT) and trunk blood (at the end of the study) was collected in tubes containing heparin and immediately centrifuged at 1000*g* for 15 min at 4°C. The resulting supernatant (plasma) was collected, aliquoted and stored at −80°C. Plasma levels of IL-6, IL-4, IL-10 IFN-γ, TNF-α and IL-1β were assayed in duplicate using high sensitivity commercially available electrochemiluminescence MULTI-SPOT® Meso Scale Discovery kits (MSD) as per the manufacturer's instructions. Reading and analysis were conducted using the MESO QuickPlex SQ 120, Sector Imager 2400. Concentration is expressed in pg/ml.

Ethics statement

The human study was approved by the Ethics Committee of 'Comitato Etico dell'IRCCS San Giovanni di Dio—Fatebenefratelli' (Brescia, Italy) under registration number 92/2017. Informed consent to participate in this study was given by all subjects or their caregivers. All animal experiments were performed in compliance with the guidelines for the welfare of experimental animals issued by the Health Products Regulatory Authority (HPRA, Ireland), and the European Communities Council Directive (2010/63/EU) and approved by the Animal Experimentation Ethics Committee (#AE19130/P108) of University College Cork.

Statistics

Sample size for experiments were determined based on prior publication and power analysis using the G*Power software. Normal distribution was determined by the Shapiro-Wilk test. For normally distributed data, single comparisons were tested using independent Student's *t*-test. Non-parametric data were examined using the Mann-Whitney U-test. Experiments with more than two groups were subjected to a one-way ANOVA if normally distributed and compared using Bonferroni's *post hoc* test. Multiple comparisons of non-parametric data were made by a Kruskal-Wallis analysis followed by Dunn's *post hoc* test. Experiments with two 'between factors' (treatment, donors) were analysed using a two-way ANOVA to determine genotype and treatment effect or interaction between

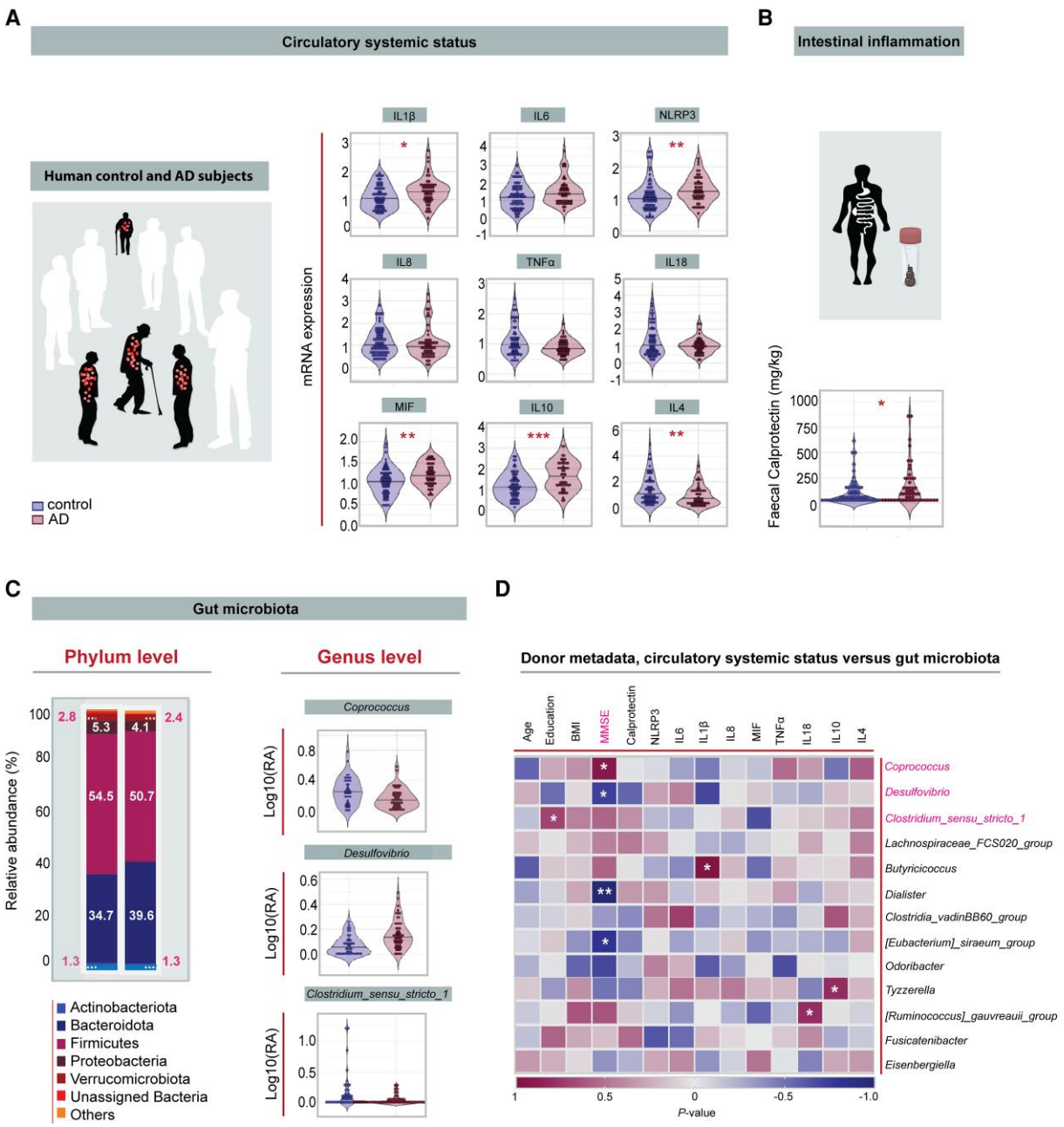

Figure 1 Associations between circulatory systemic factors, gut microbiota and cognitive status of Alzheimer's disease patients. (**A**) There was increased expression of the cytokines IL-1β (*$P = 0.018$), NLRP3 (**$P = 0.003$), MIF (**$P = 0.008$), IL-10 (***$P < 0.001$) and decreased expression of IL-4 (**$P = 0.006$) in Alzheimer's disease patients ($n = 52$) compared to control subjects ($n = 68$), unpaired, two-tailed Student's *t*-test for continuous Gaussian variables (or Mann-Whitney test for non-Gaussian variables). (**B**) Faecal calprotectin was significantly increased in Alzheimer's patients ($n = 64$) compared to control subjects ($n = 69$), Mann-Whitney test. *$P = 0.022$. (**C**) Gut microbiota composition at phylum level from control ($n = 41$) and Alzheimer's patients ($n = 54$). Alzheimer's patients had higher abundance of phyla Bacteroides, and lower abundance of phyla Firmicutes and Verrucomicrobiota. Relative abundance of genera that differed significantly between controls and Alzheimer's patients after batch correction using percentile-normalization. Mann-Whitney tests, multiple testing corrections using the Benjamini–Hochberg method, and FDR ≤ 0.1 was considered significant (*Coprococcus*, $P = 0.099$; *Clostridium in sensu stricto* 1, $P = 0.099$; *Desulfovibrio*, $P = 0.006$). (**D**) Correlation between human gut microbiota, human donor metadata, faecal calprotectin and serum markers. Heat map shows Spearman rank coefficients, with red indicating strong positive correlation, and blue indicating strong negative correlation. *P*-values for significant correlations ($\alpha < 0.05$) are noted (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Black horizontal lines in violin plots indicate medians. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS = not significant; AD = Alzheimer's disease.

both factors. Experiments with one 'between-subjects' factor (treatment group) and one 'within-subjects' factor (trial) were analysed using a two-way mixed ANOVA. Pearson or Spearman's rank correlation were performed when data were normally or not normally distributed, respectively. Statistical analyses were performed using GraphPad Prism 8.0 or in R (v.3.6.3) with the Rstudio GUI (v.1.2.5033) for bioinformatic analysis of 16S microbiota sequencing and R (v.4.1.1) for metabolomics analysis. See Supplementary material for details. Statistical significance was set at $P \leq 0.05$ and differences are indicated in the figures by *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$.

Results

Alterations in circulatory system and gut microbiota are associated with cognitive status in Alzheimer's patients

Recent studies have described a potential link between the development of Alzheimer's disease and alterations in the intestinal microbial community.[49] Dysregulation in systemic inflammatory status has long been associated with the aetiology and progression of Alzheimer's disease[50] but there is little evidence showing that alterations in gut microbiota composition are associated with circulatory systemic factors and cognitive status in Alzheimer's patients. Assessment of plasma in a cohort of 69 healthy control subjects and 64 Alzheimer's patients (Table 1) show that IL-1β, the inflammasome marker NLRP3 and the macrophage migration inhibitory factor (MIF) were significantly upregulated in Alzheimer's patients, indicating increased systemic inflammation in Alzheimer's patients (Fig. 1A). These changes were irrespective of biological sex (Supplementary material, Extended Table 6). Lipopolysaccharide (LPS), which has previously been reported to be elevated in the serum of Alzheimer's disease patients, and can influence Aβ homeostasis, tau pathology and neuroinflammation is not significantly changed in plasma of Alzheimer's disease participants compared to healthy control participants in our study (Supplementary Fig. 10) but this does not exclude the possibility that LPS may indirectly influence the brain, neurogenesis and cognition in our cohort through the release of pro-inflammatory cytokines from circulating immune cells in the plasma, as we have reported here (Fig. 1A), or through other routes, such as the brain–gut axis.[51] In addition, faecal calprotectin, which has been shown to correlate with the presence and severity of intestinal inflammation,[52] was significantly increased in Alzheimer's patients compared to control subjects (Fig. 1B). Analysis of gut microbiota composition by bacterial 16S rRNA gene sequencing in a subset of Alzheimer's patients (Supplementary Fig. 1A–E) revealed no significant change in alpha and beta diversities between control subjects and Alzheimer's patients (Supplementary Fig. 1B–D). However, at phylum level, Alzheimer's patients had a higher abundance of Bacteroidetes (Fig. 1C) reported to comprise many pro-inflammatory species,[53] and a lower abundance of the phyla Firmicutes and Verruocomicrobiota, reported to produce beneficial metabolites.[54] At the genus level, Alzheimer's patients had on average a significant reduction in the relative abundance of *Clostridium sensu stricto 1* and the short chain fatty acid (SCFA) butyrate-producing genera *Coprococcus*, which is associated with healthy ageing in general[55] (Fig. 1C). In addition, similar to a previous report using a transgenic mouse model of Alzheimer's disease,[56] the relative abundance of the pathobiont genera *Desulfovibrio* was significantly increased in Alzheimer's patients compared to cognitively healthy control subjects (Fig. 1C). There was no variability in gut microbiota composition between males and females (Supplementary material, Extended Table 7 and Supplementary Fig. 9).

To further understand whether these peripheral alterations are associated with the clinical status of Alzheimer's patients, we correlated Mini-Mental State Examination (MMSE) scores with the circulatory systemic profile and with the gut microbiota alterations. Notably, we observed significant associations between the MMSE score and the gut microbiota signature (Fig. 1D). Importantly, a positive correlation was observed between the abundance of the health-associated SCFA producer *Coprococcus* and the MMSE score, and inverse correlations were detected between the abundance of the disease-associated pathobionts *Desulfovibrio*, *Dialister* and the MMSE score, supporting a microbiome signature for cognitive performance in Alzheimer's disease.

Table 1 Demographic and clinical features of control subjects and Alzheimer's patients

	Control subjects	Alzheimer's disease subjects	*P*-value
n	69	64	–
Age, years	71.4 (7.9)	74.8 (7.3)	0.007**
Female, *n* (%)	42 (61)	34 (53)	0.367
ApoE4, *n* (%)	16 (24)	33 (58)	<0.001***
Education, years	11.3 (5.4)	7.6 (3.9)	<0.001***
BMI	25.2 (3.7)	24.7 (3.7)	0.750
Dementia score			
MMSE	29.0 (1.2)	19.8 (5.8)	<0.001***

Demographic and clinical features of control subjects ($n = 69$) and Alzheimer's patients ($n = 64$), *P*-values were calculated using unpaired, two-tailed Student's *t*-test for continuous Gaussian variables (or Mann-Whitney test for non-Gaussian variables) and chi-square test for categorical data. There were eight missing values for ApoE4, six missing values for MMSE and BMI. Alzheimer's patients were significantly older (**$P = 0.007$), showed a significant increase in the prevalence of ApoE4 carriers (***$P < 0.001$), were less educated (***$P < 0.001$) and had lower MMSE score (***$P < 0.001$) than cognitively healthy controls. Except for values on females and ApoE4, data are presented as means + SEM. A greater proportion of patients with Alzheimer's disease presented with higher incidence of hypercholesterolemia and vascular diseases and were medicated with antidepressant/anxiolytic drugs than healthy controls (Supplementary material, Extended Table 8). BMI = body mass index; MMSE = Mini-Mental State Examination.

Gut microbiota from Alzheimer's patients altered gut microbiota and intestinal epithelium in rats

To elucidate the functional contribution of human gut microbiota to Alzheimer's aetiology, we transplanted faecal samples from cognitively healthy subjects and Alzheimer's patients into microbiota-depleted young adult rat (Fig. 2A). For this purpose, cognitively healthy and Alzheimer's disease human donors were selected based on clinical features, using the MMSE and Clinical Dementia Rating (CDR) (Table 2). To validate donor engraftment and the temporal colonization dynamics of gut microbiota transplantation, we analysed baseline faecal samples using 16S rRNA gene sequencing, reassessed composition and diversity at time point 1 (T1) 10 days after human donor colonization (10d) and again at time point 2 (T2), which was the end of the study at Day 59 (59d; Fig. 2A).

Principal coordinate analysis (PCoA) of the Bray-Curtis distance matrix revealed that the temporal microbial changes were not influenced by the clinical status of the donors (Fig. 2B, top). A decrease in alpha diversity was observed in both groups of rats at both time points after human donor colonization, indicating a loss of bacterial species (Fig. 2B, bottom), which may be due to sample processing or host incompatibility. These differences were independent from the clinical status of the donors. At the end of the study (59 days after FMT), a decrease in gained taxa was observed in rats that received faecal material from Alzheimer's patients, suggesting that their microbiota tended to return to its original state (Supplementary Fig. 2C). On average, 40% of the taxa from human donors engrafted into recipient rats (Supplementary Fig. 2D). Transplantation efficiency was lowest for taxa belonging to the phyla Bacteroidota and Proteobacteria (Supplementary Fig. 2F). Post-FMT, 78 genera were significantly altered in recipient rats (Fig. 2C). Compared with the original microbiota, human Alzheimer's colonized rats displayed greater alterations in microbial genera than rats

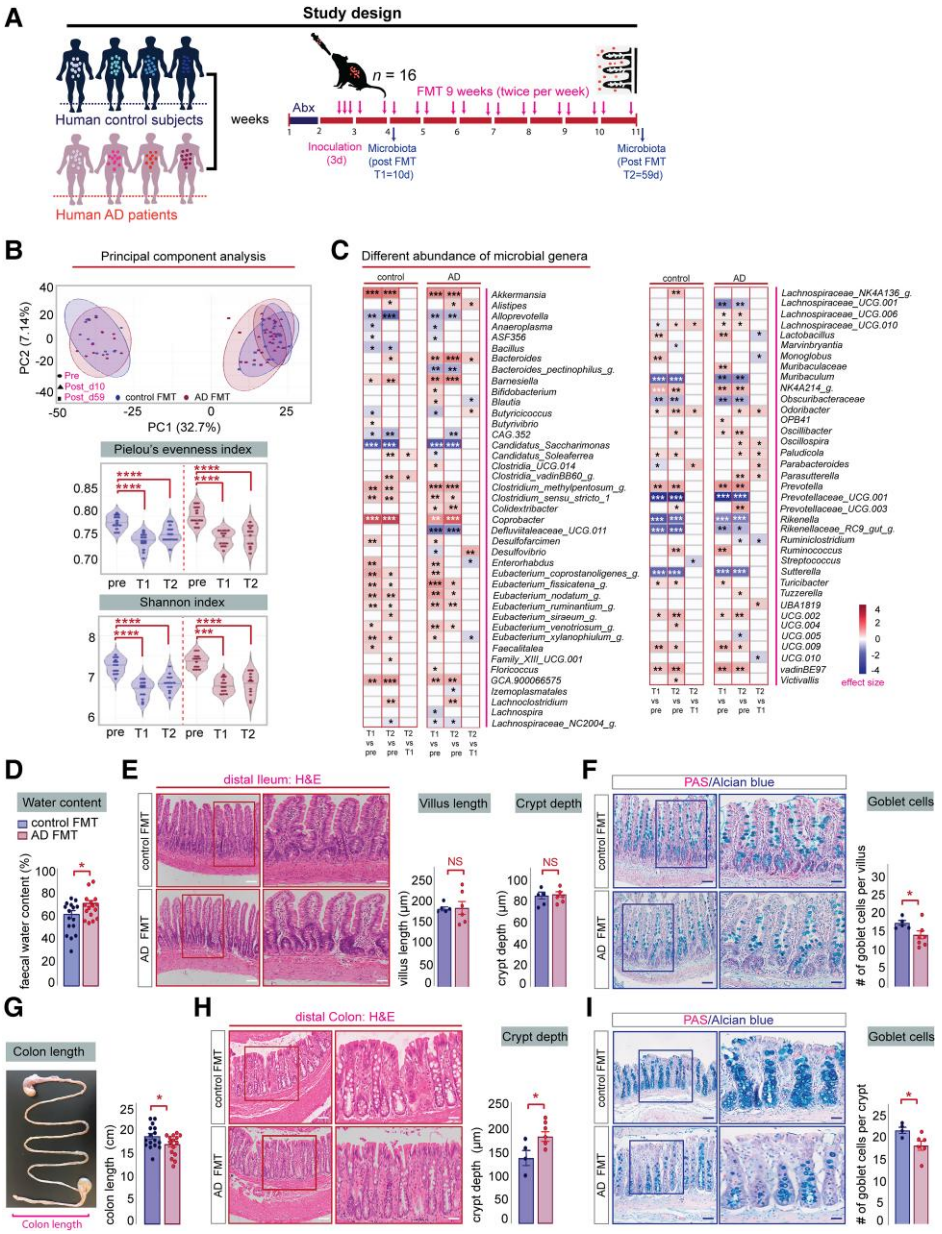

Figure 2 Adult rats colonized with faecal material from Alzheimer's patients harbour different bacterial genera and display alterations in intestinal epithelial structure. (**A**) Schematic representation of the experimental procedure: microbiota-depleted young adult rats were colonized with human faecal samples from cognitively healthy control or Alzheimer's donors ($n = 4$). One faecal sample from each human donor was used to colonize four microbiota-depleted rats ($n = 16$). Faecal pellets were collected at Day 0 (pre), 10 days after human donor colonization (10d; T1) and at the end of the study (T2 = 59 days). Vertical bars represent weekly intervals. (**B**) *Top*: Principal coordinate analysis showing the effects of FMT on faecal microbiome in rats in terms of β-diversity as measured by Bray-Curtis distance. Ellipses indicate 95% confidence intervals per group. Linear mixed-effects model, significant Time × FMT interaction effect (*$P = 0.047$). *Bottom*: Violin plots displaying the effects of FMT on rats in terms of α-diversity. Black horizontal lines in violin plots indicate medians. The Pielou's evenness and Shannon index decreased following FMT, regardless of donor group (ANOVA with repeated measures *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$). (**C**) *Left*: Heat map showing genera differentially altered by FMT. Colour depicts effect size, with blue (negative) indicating higher abundances pre-treatment and red (positive) indicating higher abundances post-treatment, Wilcoxon signed-rank test followed by Benjamini–Hochberg correction as per the ALDEx2 library, *$q < 0.1$, **$q < 0.01$, ***$q < 0.001$. (**D**) Alzheimer's-FMT rats ($n = 16$) display a significant increase in faecal water content compared to control FMT rats ($n = 16$), unpaired, two-tailed Student's *t*-test, *$P = 0.0309$. (**E**) *Left*: Representative images of haematoxylin and eosin stained sections of the distal ileum from control and Alzheimer's-FMT rats. Scale bars = 200 µm (left magnification), 100 µm (right magnification). *Right*: Alzheimer's colonized rats show no significant change in ileal villus length, unpaired, two-tailed Student's *t*-test, $P = 0.7749$ and crypt depth, unpaired, two-tailed Student's *t*-test, $P = 0.9060$. (**F**) *Left*: Representative PAS/Alcian blue staining of ileal sections. Scale bars = 200 µm (left magnification), 100 µm (right magnification). *Right*: Alzheimer's-FMT rats display significant goblet cell loss in the ileum, unpaired, two-tailed Student's *t*-test, *$P = 0.0426$. (**G**) Average colon length was significantly reduced in Alzheimer's-FMT rats compared to control FMT rats, two-tailed Student's *t*-test, *$P = 0.0500$. (**H**) *Left*: Representative images of haematoxylin and eosin stained sections of the proximal colon from control and Alzheimer's-FMT rats. Scale bars = 200 µm (left magnification), 100 µm (right magnification). *Right*: Alzheimer's-FMT rats displayed a significant increase in colonic crypt depth, two-tailed Student's *t*-test, *$P = 0.0356$. (**I**) *Left*: Representative PAS/Alcian blue staining of colon sections. *Right*: Alzheimer's-FMT rats display a significant goblet cell loss in the proximal colon, unpaired, two-tailed Student's *t*-test, *$P = 0.0403$. Unless otherwise indicated, data are presented as mean ± SEM, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **** $P < 0.0001$. AD = Alzheimer's disease; FMT= faecal microbiota transplantation; NS = not significant; PAS = periodic acid-Schiff.

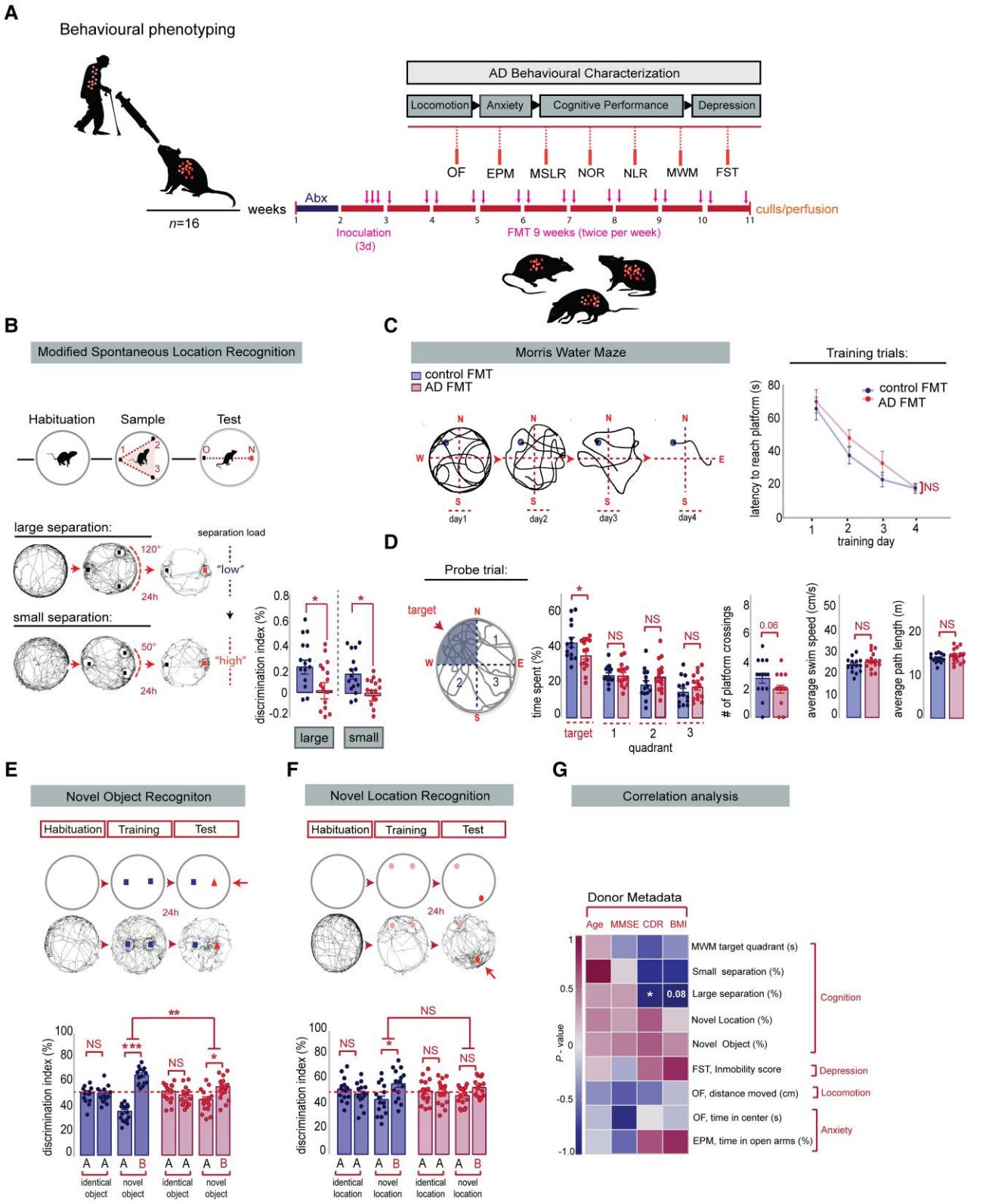

Figure 3 Alzheimer's-FMT induced cognitive deficits in hippocampal-neurogenesis dependent behaviours in young adult rats. (**A**) Overview of the Alzheimer's-related behaviours. Vertical bars represent weekly intervals. Behaviours include: Open Field (OF), Elevated Plus Maze (EPM), Modified Spontaneous Location Recognition (MSLR), Novel Object Recognition (NOR), Novel Location Recognition (NLR), Morris Water Maze (MWM) and Forced Swim test (FST). (**B**) *Top:* Illustration of the MSLR task. *Bottom:* Alzheimer's-FMT rats displayed a significant reduction in the discrimination index in the large separation task, two-tailed Student's *t*-test, $^{*}P = 0.0353$ and small separation task, Mann-Whitney U-test, $^{*}P = 0.0108$. (**C**) *Left*: Representative tracings in platform trials in the MWM. *Right*: Escape latency during platform trials in MWM. No significant differences in learning were detected during the acquisition training days in the MWM test, two-way mixed ANOVA, effect of FMT: $P = 0.2148$, effect of time: $^{***}P < 0.0001$, FMT × Time interaction: $P = 0.6672$. (**D**) When

(Continued)

Table 2 Demographic information of human donors

Donor	Sex	APOE	Age	E	BMI	MMSE	CDR
Control_1	M	ϵ3ϵ3	80	5	24.6	26	0
Control_2	F	ϵ3ϵ4	71	5	25.6	29	0
Control_3	M	ϵ3ϵ3	67	13	24.7	27	0
Control_4	M	ϵ3ϵ3	68	19	25.1	30	0
AD_1	M	ϵ3ϵ4	74	8	24.9	12	2
AD_2	F	ϵ3ϵ3	78	8	23.1	24	1
AD_3	F	ϵ3ϵ4	77	5	34.2	20	3
AD_4	F	ϵ3ϵ3	80	5	23.8	19	1

Donor metadata: demographic and clinical characteristics of human donors ($n = 4$) used for rat colonization. MMSE and CDR scores were used to determine cognitive function applicable to dementia of human donors. Cognitive function was significantly impaired in human Alzheimer's donors compared to control donors as measured by MMSE, unpaired, two-tailed Student's *t*-test, $^{*}P = 0.0131$ and CDR scores, Mann-Whitney test, $^{*}P = 0.0286$. Human donors in both groups displayed no significant difference in age, unpaired, two-tailed Student's *t*-test, $P = 0.8326$; education level, Mann-Whitney test, $P = 0.6571$ and BMI, Mann-Whitney test, $P = 0.6857$. BMI = body mass index; CDR = Clinical Dementia Rating; E = Education; MMSE = Mini-Mental State Examination.

receiving control FMT at 10d (51 genera versus 41) but not at 59d (44 versus 45) (Fig. 2C). Notably, we detected specific differences in the abundance of the genera *Desulfovibrio* in human Alzheimer's colonized rats compared to control colonized rats (Fig. 2C), which reflects our findings in Alzheimer's patients (Fig. 1C) and correlates with the MMSE score (Fig. 1D).

To elucidate whether human Alzheimer's gut microbiota induce pathophysiological processes in the gastrointestinal (GI) tract of healthy young rats, we evaluated the general health of the rat intestine after FMT. Human Alzheimer's-FMT had no significant impact on faecal pellet output, the caecum weight of recipient rats (Supplementary Fig. 3A and B), body weight composition or food intake (Supplementary Fig. 3C and D). Additionally, no significant differences were detected in the expression of genes encoding pro-inflammatory cytokines in colonic tissue of rats after FMT (Supplementary Fig. 4A–I) and the presence of cytokines in the systemic circulation was unaltered (Supplementary Fig. 5A and B), suggesting that no local inflammatory event occurred in the GI tract due to humanization with Alzheimer's-FMT. However, human Alzheimer's disease colonized rats displayed a significant increase in faecal water content (Fig. 2D) and intake (Supplementary Fig. 3E) along with a reduction in colon length (Fig. 2G), suggesting specific effects of the human Alzheimer's gut microbiota on colonic function. These findings were further supported by structural changes in the depth of the colonic but not ileal crypts as determined by haematoxylin and eosin staining (Fig. 2E and H). Compared to control colonized rats, Alzheimer's recipient rats displayed crypt hyperplasia in the proximal colon, with the depths of the crypt significantly elongated (Fig. 2H). Additionally, we found a reduction in the number of goblet cells in the colon and ileum, suggesting altered mucin production after transplantation with human Alzheimer's gut microbiota (Fig. 2F and I).

Alzheimer's FMT induced cognitive deficits in hippocampal-neurogenesis dependent behaviours in rats

To investigate whether alterations of the human gut microbiota contribute to the cause of the Alzheimer's behavioural phenotype, we evaluated behavioural performance of young adult rats colonized with faecal material from human Alzheimer's patients and age-matched control subjects. Rats were tested 2 weeks after initial human donor colonization in a behavioural test battery to assess task-specific memory performance and other Alzheimer's-related co-morbiditie (Fig. 3A). Rats colonized with faecal material from Alzheimer's donors exhibited no change in locomotor parameters in the Open Field Test (Supplementary Fig. 6A), no change in anxiety-related behaviours in the Elevated Plus Maze (EPM) (Supplementary Fig. 6B), or in antidepressant-like behaviour in the Forced Swim test (FST) (Supplementary Fig. 6C), indicating no specific effects of the Alzheimer's human gut microbiota on comorbid features of Alzheimer's disease in rats.

Given previous reports in Alzheimer's patients and transgenic animal models of Alzheimer's disease that impairments of AHN contribute to memory deficits in Alzheimer's disease,[18] we employed the modified spontaneous location recognition (MSLR) task to assess pattern separation (Fig. 3B), which has been shown to be reliant on AHN.[57] We found that rats harbouring human Alzheimer's microbiota were significantly impaired in discriminating between the familiar and novel locations in the 'large separation condition' (low cognitive load on pattern separation) and 'small separation condition' (high cognitive load on pattern separation) (Fig. 3B). Memory performance was consistently affected in a number of other cognitive tasks (Fig. 3C–F), which have also been shown to be dependent on AHN.[58-60] In the Morris Water Maze (MWM) task (Fig. 3C), humanized Alzheimer's colonized rats spent significantly less time in the target quadrant during the probe trial than control FMT-treated rats, indicating impairments in long-term spatial memory (Fig. 3D). Consistent with Alzheimer's-relevant cognitive deficits, we also found that rats colonized with material from Alzheimer's donors performed significantly worse in a novel recognition memory task (Fig. 3E) and failed to discriminate the novel location in the novel location recognition test (Fig. 3F).

Figure 3 Continued
challenged in the probe trial, Alzheimer's-FMT rats spent significantly less time in the target quadrant, unpaired, two-tailed Student's *t*-test, $^{*}P = 0.0498$. Average number of platform crossings was reduced in Alzheimer's-FMT rats compared to control FMT rats, Mann-Whitney test, $P = 0.0670$. Alzheimer's-FMT colonized rats displayed no significant change in the locomotory parameters of the probe trial, as determined by average swim speed, unpaired, two-tailed Student's *t*-test, $P = 0.1582$ and path length, unpaired, two-tailed Student's *t*-test, $P = 0.2246$. (**E**) *Top*: Illustration of the NOR test. *Bottom*: In the training session, no significant difference was detected between the differentiation index of the two identical objects in the control FMT rats, two tailed paired Student's *t*-test, $P = 0.9859$ and Alzheimer's-FMT rats, $P = 0.4149$. In the test session, Alzheimer's-FMT rats show a significant reduction in the discrimination index of the novel objects compared to control FMT rats, unpaired, two-tailed Student's *t*-test, $^{**}P = 0.0019$. (**F**) *Top*: Illustration of the NOL test. *Bottom*: In the training sessions, no significant difference was detected between the differentiation index of the two identical locations in the control FMT rats, two tailed paired Student's *t*-test, $P = 0.4244$ and Alzheimer's-FMT rats, $P = 0.8267$. In the test session, Alzheimer's-FMT rats show no significant reduction in the discrimination index of the novel location compared to control FMT rats, unpaired, two-tailed Student's *t*-test, $P = 0.4032$. (**G**) Spearman's rank and Pearson correlation between rat behaviour and human donor metadata. Heat map showing Spearman *p* (rho) or Pearsons R correlation coefficients, with red indicating strong positive correlation, and blue indicating strong negative correlation. *P*-values for significant correlations ($\alpha<0.05$) are noted. All data are presented as mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. AD = Alzheimer's disease; BMI = body mass index; CDR = Clinical Dementia Rating; FMT= faecal microbiota transplantation; MMSE = Mini-Mental State Examination; NS = not significant.

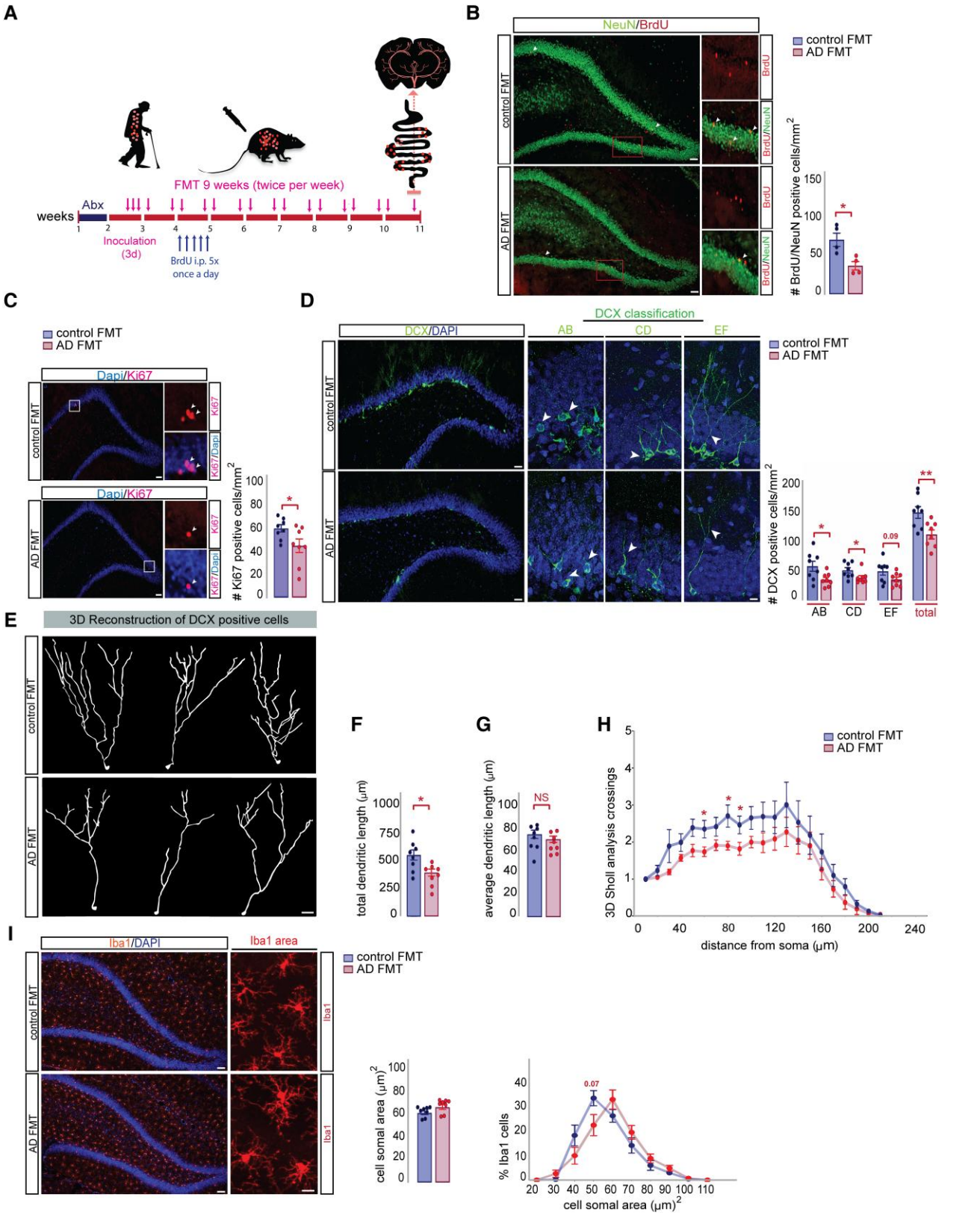

Figure 4 Transplantation of gut microbiota from Alzheimer's patients decreased neurogenesis and dendritogenesis of adult-born hippocampal neurons in rats. (**A**) Experimental timeline showing the effect of FMT on the survival of newborn neurons and microglia activation: rats were injected with BrdU (150 mg/kg) once per day for 5 days, 10 days post donor colonization and sacrificed 6 weeks later at Day 59. (**B**) *Left*: Representative images of BrdU/NeuN positive cells in DG of control and Alzheimer's-FMT rats. Scale bars = 200 µm (left magnification), 50 µm (right magnification). Newborn neurons were analysed using double labelling for BrdU (red) and the neuronal marker NeuN (green). Arrowheads indicate double-positive cells (orange). *Right*: Alzheimer's-FMT rats show a significant reduction in the number of BrdU/NeuN cells, two-tailed Student's *t*-test, $^{*}P = 0.0159$. (**C**) *Left*: Representative

(continued)

These observations were further corroborated by correlating the clinical human donor profile to the Alzheimer's behavioural readouts of the recipient rats. We found an inverse correlation between the discrimination index of the MSLR (large separation) and the CDR of the human donors (Fig. 3G), supporting the hypothesis that dementia donor-specific microbiota changes impact on cognitive function. Overall, our results demonstrate that the presence of human gut microbiota from Alzheimer's patients promotes cognitive deficits related to the clinical symptoms of Alzheimer's disease and impairs, in particular, AHN-dependent memory performance in recipient rats.

Transplantation of gut microbiota from Alzheimer's patients decreased neurogenesis in adult rats

Based on these results and given the well documented role of the dentate gyrus (DG) in supporting pattern separation, we assessed AHN in the rat DG after human FMT (Fig. 4A). We measured the survival of new neurons in the DG by performing BrdU labelling and co-localization with the neuronal marker NeuN 6 weeks after injection of BrdU (Fig. 4B). A significant decrease in the number of BrdU/NeuN positive cells in the DG of rats colonized with Alzheimer's faecal material compared to control recipient rats was evident, indicating reduced survival of new neurons in the DG (Fig. 4B). In line with these findings, we observed that the number of Ki67 (Fig. 4C) and DCX-positive cells was significantly lower in Alzheimer's colonized rats (Fig. 4D). This density reduction was specific to AB-type (proliferative, short or no dendrites) and CD-type (intermediate, dendrites reaching molecular layer) cells but not EF-type (postmitotic, multi-branched dendrites) cells.[61]

New neurons produced in the hippocampus of the DG have been shown to functionally integrate into mature circuits and contribute to the ability to recall memories. One requirement, however, for the successful synaptic integration and subsequent functionality of these neurons is the dendritic sprouting and proper arborization into the molecular layer of the DG.[62] To gain insight into the mechanism that may contribute to the observed memory deficits in Alzheimer's colonized rats, we carried out 3D-reconstruction of DCX-positive cells using Sholl analysis to evaluate the complexity of their dendritic trees (Fig. 4E). Compared to control colonized rats, DCX labelled cells in Alzheimer's-FMT treated rats displayed a significant reduction in the total dendritic length, whereas the average dendritic length per neuron was unaltered (Fig. 4F and G). When we quantified the number of dendritic intersections, we observed a significant reduction of dendritic complexity in Alzheimer's colonized rats compared to control recipient rats (Fig. 4H). These data indicate that gut microbiota transferred from Alzheimer's disease negatively affect the survival and dendritic arborization of adult-born neurons into the molecular layer of the DG of the rat.

Rats harbouring gut microbiota from Alzheimer's patients did not show significant differences in microglia density in the DG (Supplementary Fig. 7A) and showed only minor differences in Iba1 somal size (Fig. 4I and Supplementary Fig. 7B) compared to control FMT colonized rats, suggesting that neuroinflammatory processes in this brain region may have a minimal role in the cognitive impairments observed in recipient rats colonized with microbiota from Alzheimer's patients. To examine whether the impaired cognitive function in Alzheimer's colonized rats might be related to plaque formation, we performed Thioflavin-S fluorescent microscopy of coronal brain sections, which revealed the absence of plaque deposition in the hippocampus and cortex in both groups (Supplementary Fig. 7C and D). Overall, these data reinforce previous findings in post-mortem human brains that cognition and AHN is already altered before extracellular amyloid deposition.[18]

Serum from Alzheimer's patients decreased neurogenesis in human hippocampal progenitor cells *in vitro*

Since the hippocampal neurogenic niche is highly vascularized[63-65] and gut microbiota composition explains up to 58% of the variance of individual plasma metabolites,[66] Alzheimer's gut microbiota-induced changes in the systemic environment, mediated by blood, have the potential to modulate AHN. Given the inaccessibility of the DG and the absence of validated biomarkers and neuroimaging tools to quantify hippocampal neurogenesis in living humans, we used our *in vitro* neurogenesis assay to study how the Alzheimer's systemic environment modulates hippocampal neurogenesis[67-70] (Fig. 5). To that end, embryonic human hippocampal progenitor cells (HPCs) were exposed to serum from control subjects and Alzheimer's patients (Fig. 5A) and the percentage of cells expressing markers for neural stem cell proliferation (Ki67), differentiation (MAP2, DCX), and programmed cell death (CC3) was determined.[71] Our results show that, while the average cell density of control and Alzheimer's serum exposed HPCs cells remained unaffected (Fig. 5B), a reduction in the expression of Ki67 positive cells (Fig. 5B) in response to Alzheimer's serum occurred, indicating that

Figure 4 (Continued)
images of Ki67 positive cells in DG of control and Alzheimer's-FMT rats. Scale bars = 200 µm (left magnification), 50 µm (right magnification). *Right*: A significant reduction in the number of Ki67 positive cells in rats after FMT from Alzheimer's disease, two-tailed Student's *t*-test, $^{*}P = 0.0411$. (**D**) *Left*: Representative images of AB, CD and EF types of DCX-positive cells classified based on their dendritic tree morphology. Scale bars = 200 µm (left magnification), 40 µm (right magnification). *Right*: Alzheimer's-FMT rats show a significant reduction in the number of AB type, two-tailed Student's *t*-test, $^{*}P = 0.0290$; CD type, Mann-Whitney test, $^{*}P = 0.0281$ and total number of DCX-positive cells in the DG, two-tailed Student's *t*-test, $^{**}P = 0.0099$. (**E**) Representative 3D reconstructions of DCX-positive cells from the DG of young adult rats after control and Alzheimer's-FMT. Scale bar = 20 µm. (**F**) Morphological analysis revealed a significant decrease in the total dendritic length of DCX-positive cells in Alzheimer's-FMT treated rats, two-tailed Student's *t*-test, $^{*}P = 0.0124$ (**G**) The average dendritic length of DCX-positive cells was unaltered between control and Alzheimer's-FMT rats, two-tailed Student's *t*-test, $P = 0.3533$. (**H**) Sholl analysis revealed a reduction in dendritic complexity of DCX-positive cells in Alzheimer's-FMT rats compared to control FMT rats, two-way mixed ANOVA, effect of FMT: $^{*}P = 0.0452$, effect of distance from soma: $^{***}P < 0.0001$, FMT × Distance interaction: $P = 0.9358$. (**I**) *Left*: Representative images of Iba1 positive cells in the DG of control and Alzheimer's-FMT rats. Scale bars = 200 µm (left magnification), 40 µm (right magnification). *Right*: Quantification of cell soma area of Iba1-positive cells in the DG of control and Alzheimer's-FMT rats, Mann-Whitney test, $P = 0.1605$. Distribution analysis of soma area shows a shift from small to larger cell body sizes in Alzheimer's-FMT colonized rats in comparison to control FMT colonized rats. Two-way mixed ANOVA, effect of FMT: $P = 0.3154$, effect of soma area: $^{***}P < 0.0001$, FMT × Soma area interaction: $^{*}P = 0.0240$. All data are presented as mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$. AD = Alzheimer's disease; DG = dentate gyrus; FMT = faecal microbiota transplantation; NS = not significant.

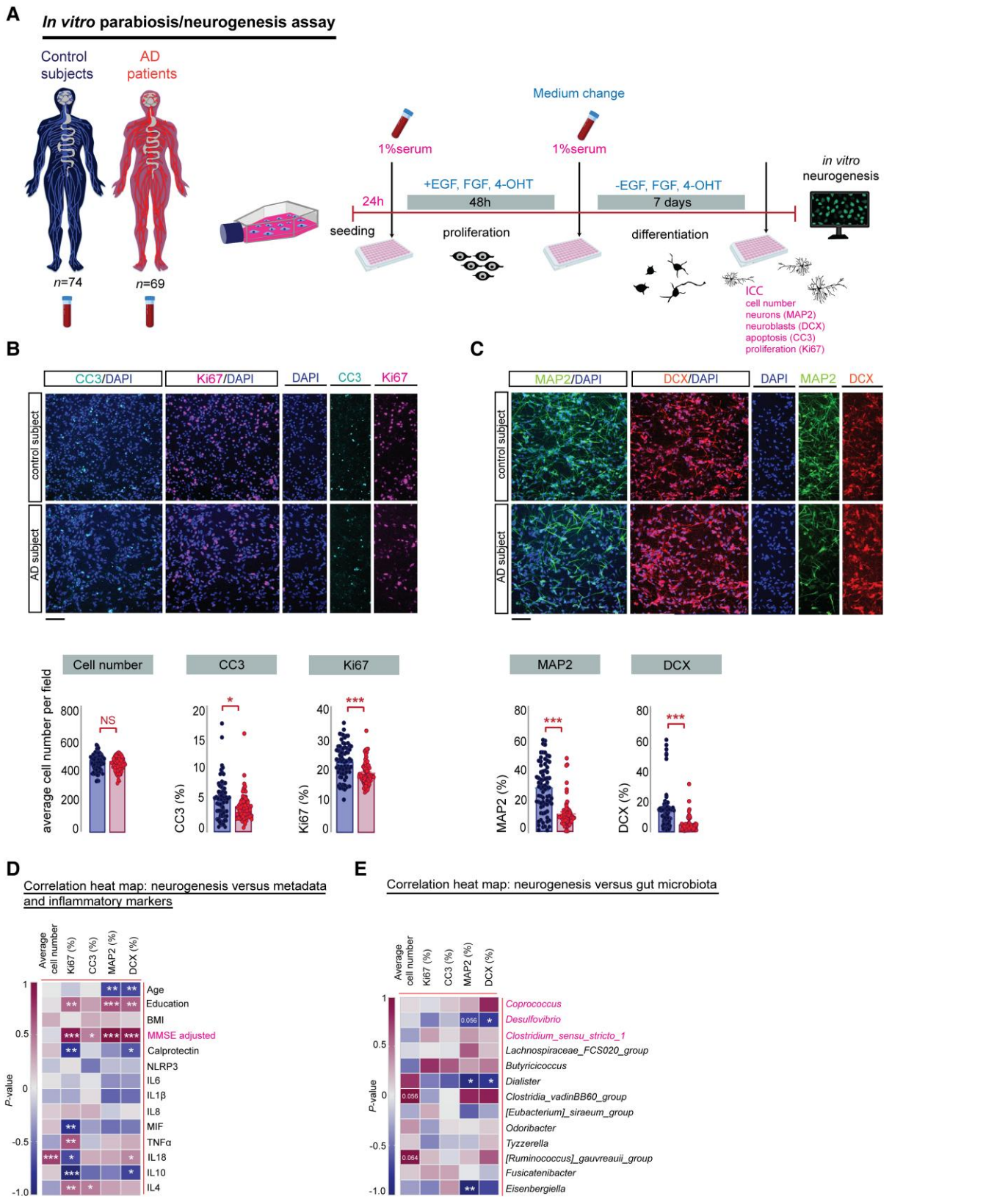

Figure 5 Serum from Alzheimer's patients decreased neurogenesis in human hippocampal progenitor cells. (**A**) Experimental timeline of the *in vitro* parabiosis/neurogenesis assay: human hippocampal progenitor cells (HPCs) were cultured with 1% human serum from age-matched cognitively healthy and Alzheimer's patients. Cellular readouts are expressed as percentage relative to neural cell number. Fifteen fields were analysed per well, $n = 3$ biological replicates. (**B**) *Top*: Representative confocal micrographs showing CC3-positive HPCs cells (green: CC3-positive cells, blue: DAPI) and Ki67-positive HPCs cells (red: DCX-positive cells, blue: DAPI) during the differentiation phase of the assay. Scale bar = 100 μm. *Bottom*: No significant change was detected in the average number of HPCs per field between control and Alzheimer's patients during the differentiation phase of the human

(continued)

the serum of Alzheimer's patients decreases the proliferative capacity of differentiating HPCs. Furthermore, following 7 days of neuronal differentiation, we observed a decrease in the percentage of MAP2 and DCX positive neuroblasts in Alzheimer's serum-treated cells (Fig. 5C), suggesting that the serum of Alzheimer's patients impaired neuronal differentiation. Similar to previous reports using an Alzheimer's disease transgenic mouse model,[72] we found that neuronal HPCs treated with Alzheimer's serum displayed a substantially different morphology, characterized by an increase in the total neurite length and highly increased branching points in both MAP2 and DCX-positive cells (Supplementary Fig. 8B and C) compared to HPCs exposed to control serum. Overall, these data highlight that the systemic, circulatory environment of Alzheimer's patients is capable of affecting neuronal proliferation, differentiation and dendritic morphology.

Notably, we could again link these observations to the clinical phenotype of Alzheimer's patients. We found a significant positive association between the MMSE score (Fig. 5D) and the prevalence of DCX, Ki67 and MAP2 cells. Additionally, in line with our previous findings in rats (Fig. 2E), the abundance of the parabiont genera *Desulfovibrio* and *Dialister* inversely correlated with the *in vitro* expression of the neuronal marker DCX (Fig. 5E). The association of specific bacterial genera with markers for neurogenesis, which are also highly correlated with the MMSE score (Fig. 2E), further support the hypothesis that impaired neurogenesis may be the converging link between the observed altered gut microbiota composition and cognitive impairment in Alzheimer's disease.

Recipient rats transplanted with human Alzheimer's gut microbiota show discrete metabolomic profiles

To better understand which factors may trigger the observed effects of gut microbiota transferred from Alzheimer's patients on AHN and associated behaviours, we performed untargeted metabolomic analyses of caecal content and hippocampal tissue from control and Alzheimer's colonized rats. Partial least squares discriminant analyses suggested an effect of donor status on the caecal (Fig. 6A) and hippocampal (Fig. 6B) metabolome. Indeed, differential expression analyses revealed that 13 (out of 184) metabolites in the caecal content (Fig. 6C and E) and three (out of 123) metabolites in the hippocampus (Fig. 6D and F) were nominally ($P < 0.05$) differentially abundant in Alzheimer's-FMT compared to control FMT-colonized rats (for a full list of quantified features, see Supplementary material, Extended Table 4). It should be noted however that metabolic features in either dataset did not pass the 5% false discovery rate (FDR)-based correction for multiple testing.

In caecal content, the amino acid histidine and its derivative acetylhistidine were nominally altered, in line with previous reports on peripheral metabolic profiles of both Alzheimer's patients and transgenic Alzheimer's disease mouse models.[73-77] Increased levels of histidine may indicate perturbed homeostasis of histamine,[73] which has been shown to influence AHN as well blood–brain barrier integrity.[78] Additionally, histidine plays a critical role in the membrane binding and neurotoxicity of Aβ fibrils.[79,80] Aminoadipic acid was found to be upregulated, and this has also been found in the plasma metabolome of people with Alzheimer's and mild cognitive impairment compared to age- and sex-matched controls.[81] Galactonic acid and succinic acid (nominally decreased and increased, respectively, in Alzheimer's-FMT rats) were found in one study to be increased in the urine metabolome of presenilin double knockout mice, and this was correlated with gut microbiota composition changes.[82] The tryptophan metabolites kynurenic acid and xanthurenic acid were upregulated in Alzheimer's recipient rats, which have been found to be altered in Alzheimer's serum, urine and CSF.[83] Increases in faecal concentration of the carboxylate tetradecanedioic acid, found upregulated here, have been closely linked to ageing and the prediction of neurocognitive disorders.[84,85] We also detected decreased levels of the metabolite hydroxybutyrate. Beta-hydroxybutyrate mitigates against the pathogenesis of Alzheimer's disease through various mechanisms, including through mitochondrial metabolism, metabolism of Aβ and tau proteins, inhibition of inflammation, promotion of AHN and through the regulation of cognitive function.[38,86]

While many of the above metabolites can cross the blood–brain barrier, they were not differentially abundant in the hippocampus. In the hippocampus, 4-guanidinobutyric acid and taurine were nominally downregulated. These metabolites were similarly found to be decreased in an age-dependent manner in the hippocampi of Alzheimer's disease (3xTg) model mice.[87] Critically, taurine has been found to regulate adult[88] and developmental[89] AHN, as evidenced both *in vivo* and *in vitro*. Homocitrulline was also found to be downregulated. Interestingly, (homo)citrullination of Alzheimer's-related proteins in the human medial temporal lobe proteome has been found to be altered in early Alzheimer's disease compared to healthy age-matched controls.[90] Additionally, citrulline is increased in the plasma of people with Alzheimer's[91] and the hippocampus of Alzheimer's disease model (3xTg) mice.[92]

Discussion

In this study, we demonstrate that the transplantation of human gut microbiota from Alzheimer's patients is sufficient to produce core cognitive symptoms of Alzheimer's disease coupled with an impairment in AHN, in healthy young adult rats. Moreover, application of human Alzheimer's disease serum provoked an impairment in AHN in human cells *in vitro*, supporting AHN as a converging cellular process regulating systemic circulatory and gut-mediated factors in Alzheimer's disease.

Figure 5 (Continued)
serum assay, unpaired, two-tailed Student's *t*-test, $P = 0.1855$. Staining for the apoptotic marker CC3 revealed a significant decrease in cell death in HPCs cells receiving serum from Alzheimer's patients compared to controls, Mann-Whitney test, $^{*}P = 0.0100$. Serum from Alzheimer's patients induced a significant reduction in the expression level of the proliferation cell marker Ki67, Mann-Whitney test, $^{***}P < 0.0001$. (**C**) *Top*: Immunofluorescence staining of MAP2-positive HPCs cells (green: MAP2-positive cells, blue: DAPI) and DCX-positive HPCs cells (red: DCX-positive cells, blue: DAPI) during the differentiation phase of the assay. Scale bar = 100 μm. *Bottom*: Serum from Alzheimer's patients caused a significant reduction in the expression levels of neurons (MAP2), Mann-Whitney test, $^{***}P < 0.0001$ and neuroblasts (DCX), Mann-Whitney test, $^{***}P < 0.0001$. (**D**) Spearman's rank correlation between *in vitro* cellular neurogenesis readouts, human donor metadata and serum inflammatory markers. Correlation heat maps showing Spearman's rank coefficients, with red indicating strong positive correlation and blue indicating strong negative correlation. (**E**) Spearman correlation between *in vitro* cellular neurogenesis readouts and human donor gut microbiota. *P*-values for significant correlations ($\alpha < 0.05$) are noted. Control serum $n = 74$, Alzheimer's serum $n = 69$. All data are presented as mean ± SEM. Panel (**A**) was created with BioRender.com. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. AD = Alzheimer's disease; ICC = immunocytochemistry.

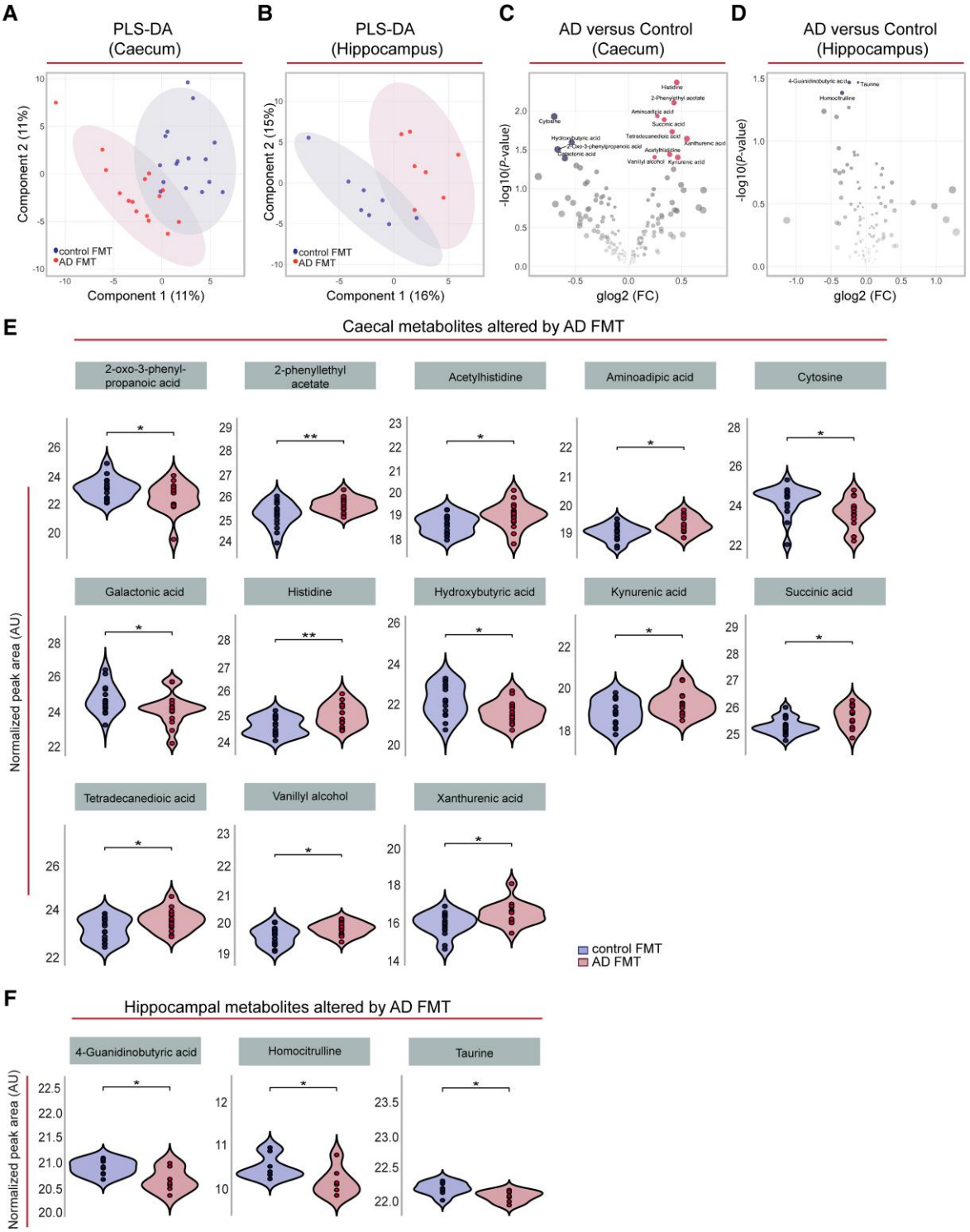

Figure 6 FMT from Alzheimer's patients induced alterations in the caecal and hippocampal metabolomes of young adult rats. (**A** and **B**) Partial least squares discriminant analysis (PLS-DA) plot depicting the effect of control and Alzheimer's-FMT on the caecal content (**A**) and hippocampal (**B**) metabolomes of young adult rats (with 95% concentration ellipses). Both models were non-significant ($pQ^2 > 0.05$) by $n = 1000$ permutation tests. (**C** and **D**) Volcano plots of metabolites quantified in caecal content ($n = 184$ features) (**C**) and hippocampal tissue ($n = 123$ features) (**D**) of rats colonized with control and Alzheimer's-FMT. Red dots represent metabolites nominally ($P < 0.05$) upregulated in Alzheimer's-FMT rats, and blue dots represent metabolites nominally ($P < 0.05$) upregulated in control FMT rats. (**E** and **F**) Violin and box-and-whisker plot (median represented by horizontal line) of normalized peak area of the caecal ($n = 15$–16) and hippocampal ($n = 7$–8) metabolites differentially regulated between rats receiving human FMT from control subjects and Alzheimer's patients, as depicted in **C** and **D**. *$P < 0.05$, **$P < 0.01$ (unadjusted *P*-values from moderated *t*-tests, Limma). Histidine, $P = 0.0043$; 2-phenylethyl acetate, $P = 0.0079$; aminoadipic acid, $P = 0.012$; cytosine, $P = 0.012$; succinic acid, $P = 0.013$; tetradecanedioic acid, $P = 0.019$; xanthurenic acid, $P = 0.023$; hydroxybutyric acid, $P = 0.026$; 2-oxo-3-phenylpropanoic acid, $P = 0.031$; acetylhistidine, $P = 0.036$; vanillyl alcohol, $P = 0.039$; kynurenic acid, $P = 0.040$; galactonic acid, $P = 0.041$. 4-guanidinobutyric acid, $P = 0.034$; taurine, $P = 0.034$; homocitrulline, $P = 0.041$. AD = Alzheimer's disease; FC = fold change; FMT = faecal microbiota transplantation; $glog_2$ = generalized logarithm base 2.

The results of the present study confirm that Alzheimer's disease is characterized by systemic and gut inflammation.[29,93] Moreover, the decrease in abundance of the phyla Firmicutes and the increase in Bacteroidetes observed in Alzheimer's patients compared with age-matched cognitively healthy subjects is in line with several findings in US[35] and Chinese populations.[34,36] At genera level and in agreement with our findings, reduced abundance of *Clostridium sensu stricto 1* is associated with adverse outcomes in Alzheimer's disease.[33,35] The parabiont *Desulfovibrio* has also been found to be enriched in other Alzheimer's cohorts[33,94] and is associated with reduced caecal levels of SCFAs and with inflammation in mice.[95] As previously reported, we found a lower proportion of bacteria with the potential to synthetize butyrate,[33,94] a microbial metabolite negatively associated with cortical amyloid accumulation.[96] Furthermore, we confirmed reduced abundance of the genus *Coprococcus* in Alzheimer's disease,[33] which is associated with amyloid accumulation.[97] When interpreting microbiota data however, it is important to keep in mind that results may be influenced by geography,[98] the inclusion criteria of study participants and methodological difference (i.e. sample collection and processing, freeze-thaw, storage methods, different bioinformatics pipelines).[99,100] Not surprisingly, human faeces from different donors engrafted in rats at different rates. Notwithstanding, transfer of faecal microbiota from cognitively healthy subjects to rats resulted in the taxa diversity remaining relatively stable over time, whereas after transfer from Alzheimer's donors, there was a greater alteration in taxa between 10 and 59 days post-FMT. Notably, one of the genera that was increased at Day 59 post Alzheimer's-FMT compared to Day 10 was *Desulfovibrio*, a genus that was also significantly enriched in Alzheimer's participants. It should be noted that healthy and Alzheimer's disease FMT donors were not balanced by biological sex, a factor that may influence the gut microbiota.[101] Although no biological sex-associated differences emerged in the gut microbiota analysis (Supplementary Fig. 9), future research investigating the role of biological sex in gut microbiota diversity in the context of Alzheimer's disease is necessary. Factors such as diet, physical activity, stress and other environmental influences, such as medication use, can also influence the composition of the gut microbiota. Thus, comprehensive assessments in future studies of health status, lifestyle factors, medication history and other factors, which specifically shape the gut microbiota, will inform potential implications for disease progression, management and the development of strategies to prevent or delay cognitive decline through modulation of the gut microbiota.

To determine whether the changes in the gut microbiota composition in Alzheimer's patients precipitate symptoms associated with the disease, we performed a series of behavioural tests on young adult rats colonized with faecal material from cognitively healthy or Alzheimer's subjects. We found a reduction in pattern separation, a hippocampal neurogenesis-reliant memory process, which is frequently reported to be impaired in Alzheimer's disease[15,17] and is correlated with indicators of pathology, such as increased cortical Aβ burden,[102] medial temporal lobe tau,[103] CSF levels of phosphorylated tau,[104] and apolipoprotein E genotype.[105] In addition, we demonstrated that Alzheimer's-FMT induced impairment in long-term spatial memory in the MWM, which is also associated with AHN levels.[58] This is in line with a recent intra-species report using transfer of a faecal sample from the 5xFAD mouse model of Alzheimer's disease into wild-type recipient mice.[39] We further demonstrate that recognition memory was impaired in Alzheimer's-FMT rats, which agrees with previous work showing an impairment in germ-free mice that received FMT from one Alzheimer's patient.[106] While alterations in the gut microbiome have been associated with altered behaviour, such as hippocampal-dependent cognition during ageing,[33] there is no evidence to date of transfer of Alzheimer's-associated cognitive behaviour to a healthy young organism via gut microbiota to confirm a causal role of gut microbiota in Alzheimer's disease, such as we have shown here.

Interestingly, the memory impairments we observed in young adult rats induced by gut microbiota from Alzheimer's patients are reliant on AHN. Our analysis of AHN in recipient rats revealed that the survival of new neurons and the number of newly born neurons was decreased after Alzheimer's-FMT, which supports our behavioural observations. We also found that rats humanized with Alzheimer's-FMT displayed an impairment in the morphological development of these newborn neurons, rendering a delay or a defect in this critical process of enabling functional integration into hippocampal circuits.[107,108] In line with our findings, the dendritic complexity of immature neurons was deficient in a familial Alzheimer's mouse model[109] and intra-species FMT (5xFAD to wild-type mice) reduced the survival and proliferation of neurons.[39] It has recently been reported that reconstitution of gut microbiota in antibiotic-treated newborn mice via FMT prevented the decrease in AHN and cognitive deficits in later life in these mice,[110] suggesting that AHN is a cellular target modifiable by gut microbiota for potential therapeutic gain. Coupled with mounting evidence of a role for the microbiota–gut–brain axis in regulating AHN, and that impairments in AHN contribute to Alzheimer's symptoms,[19] our results highlight AHN as a critical process mediating cognitive changes impacted by gut microbiota in Alzheimer's disease.

In line with our findings in rats *in vivo*, we observed decreased neurogenesis in human-derived HPCs treated with blood serum from Alzheimer's patients; we found a lower level of DCX-positive neuroblasts, Map2-positive immature neurons and proliferating HPCs. These findings are in agreement with studies that report fewer immature neurons in Alzheimer's patients compared to cognitively healthy controls.[18,111-113] Intriguingly, we previously found that an increased baseline cell death during differentiation predicts cognitive decline over the subsequent 12 years in cognitively healthy subjects,[68] whereas, here, we observed a decrease in cell death after treatment with Alzheimer's serum, suggesting that there is a differential effect of Alzheimer's serum on cell death during the disease course. Neurite outgrowth is a critical step in the formation of new neurons and integration into the hippocampal circuitry.[114] When we examined how the Alzheimer's systemic environment influences neurite outgrowth *in vitro*, complexity measures were increased after Alzheimer's serum treatment. Our findings are in line with a study that showed enhanced neurite outgrowth and arborization in a primary neuronal culture from transgenic *Drosophila melanogaster* larvae expressing human $A\beta_{42}$ compared to control,[115] which occurred prior to the Alzheimer's pathology. Surprisingly, our findings of longer and more complex neurites under Alzheimer's serum conditions *in vitro* contrasts with our observations in the rat DG. One of the reasons for the opposing finding in the Alzheimer's-FMT adult rat HPCs and the Alzheimer's-serum treated embryonic human HPCs *in vitro* could be the difference in maturation rates of HPCs between immortalized foetal human HPCs and adult rat HPCs. It has been demonstrated that adult HPCs exhibit various molecular and cellular properties that differ from those present in developmental HPCs including more restricted multipotency, acquisition of a quiescence state and different transcriptomic programmes underlying those cellular processes.[116] When we investigated the association

between *in vitro* neurogenic readouts and the gut microbiota composition of the serum donors, we found that the percentage of DCX-positive neuroblasts inversely correlated with parabiont genera *Desulfovibrio* and *Dialister*, and the percentage of Map2-positive cells inversely correlated with genera *Desulfovibrio*, *Dialister* and *Eisenbergiella*. This is interesting considering a previously published observation that genus *Eisenbergiella* increases with severity of cognitive impairment[117] and that genus *Desulfovibrio* was negatively correlated with MMSE scores.[118,119] Furthermore, *Desulfovibrio* has been found to be increased by a Western style diet and by a high fat diet but decreased by endurance exercise,[120,121] in mice suggesting susceptibility to lifestyle factors on its relative abundance in the gut. Conversely, the relative abundance of the parabiont genus *Eisenbergiella* has been reported to be decreased by a Western style diet[122] and increased in a model of stress in rats[123] as well as in adult male athletes[124] indicating a complex relationship between lifestyle factors and the host microbiome, which may influence cognitive function and the risk of cognitive decline, possibly through changes in hippocampal neurogenesis. In a previous study involving cognitively healthy individuals, we demonstrated the association between altered apoptosis, reduced HPC integrity, and lifestyle factors such as exercise and diet. These findings were further linked to future cognitive decline and dementia.[68] Additionally, we recently demonstrated the predictive value of neurogenesis assay readouts, combined with years of education, in identifying individuals at risk of developing Alzheimer's disease before clinical diagnosis.[125] Future studies will aim to validate and expand upon these observed correlations between neurogenesis assay readouts and specific microbiota genera, by investigating the underlying mechanisms by which these genera influence neurogenesis and their potential implications for Alzheimer's disease pathogenesis. Longitudinal studies are also warranted to assess whether these correlations persist over time and to determine if they have any predictive value for Alzheimer's disease development or progression. Finally, intervention studies targeting specific microbiota genera could help elucidate the causal relationship between the microbiota, neurogenesis and Alzheimer's disease, potentially opening avenues for novel therapeutic approaches.

The composition of the human gut microbiota has been found to account for a significant amount of variation in individual circulating metabolite levels.[66] We found alterations in the caecal and hippocampal metabolomes of young adult rats colonized with Alzheimer's-FMT compared to control FMT. The majority occurred in the caecum, but these were not reflected in the hippocampus, suggesting they may not directly influence AHN. The limited amount of differential expression and low effect sizes may be due to the variable levels of engraftment (i.e. by donor and genera) and reversion of microbial signatures with time, and thus did not allow for pathway analyses. Notably, however, the free amino acid taurine was decreased in the hippocampus of Alzheimer's-FMT rats. Taurine administration has repeatedly been shown to increase hippocampal neural stem and progenitor cell proliferation, survival and neurogenesis both *in vivo* and *in vitro*.[88] Given its blood–brain barrier permeability,[126] it is conceivable that some of the observed effects of Alzheimer's-FMT on AHN could partly be mediated via taurine. The current data suggest that microbial metabolites could impact both cognitive function and Alzheimer's pathology, possibly linking specific microbial signatures to the observed changes in hippocampal neurogenesis and associated cognitive behaviours. Future studies could incorporate metabolomic analyses of both donor and recipient sera to investigate gut microbiota-metabolite associations moderated by case-control status.

In conclusion, our results demonstrate that colonization of healthy young adult rats with gut microbiota from Alzheimer's patients induced behavioural and neurogenic alterations typical of Alzheimer's disease. We show that the expression of caecal metabolites involved in the neurogenic and cognitive function are altered after FMT from Alzheimer's patients, and report a direct and negative impact of serum from Alzheimer's patients on neurogenesis *in vitro*. Overall, our findings reveal that Alzheimer's symptoms can be transferred to a healthy young organism via the gut microbiota, confirming a causal role of gut microbiota in Alzheimer's disease. Furthermore, AHN is established as a converging central cellular process for cognitive changes influenced by both systemic circulatory and gut-mediated factors in Alzheimer's disease.

Data availability

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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