



Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat



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ABSTRACT

The gut microbiota interacts with the host via neuroimmune, neuroendocrine and neural pathways. These pathways are components of the brain-gut-microbiota axis and preclinical evidence suggests that the microbiota can recruit this bidirectional communication system to modulate brain development, function and behaviour. The pathophysiology of depression involves neuroimmune-neuroendocrine dysregulation. However, the extent to which changes in gut microbiota composition and function mediate the dysregulation of these pathways is unknown. Thirty four patients with major depression and 33 matched healthy controls were recruited. Cytokines, CRP, Salivary Cortisol and plasma Lipopolysaccharide binding protein were determined by ELISA. Plasma tryptophan and kynurenine were determined by HPLC. Fecal samples were collected for 16s rRNA sequencing. A Fecal Microbiota transplantation was prepared from a sub group of depressed patients and controls and transferred by oral gavage to a microbiota-deficient rat model. We demonstrate that depression is associated with decreased gut microbiota richness and diversity. Fecal microbiota transplantation from depressed patients to microbiota-depleted rats can induce behavioural and physiological features characteristic of depression in the recipient animals, including anhedonia and anxiety-like behaviours, as well as alterations in tryptophan metabolism. This suggests that the gut microbiota may play a causal role in the development of features of depression and may provide a tractable target in the treatment and prevention of this disorder.

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1. Introduction

Accumulating evidence from preclinical studies suggests that the gut microbiota can modulate brain activity and behaviour via neuroendocrine, neuroimmune, neural and humoral pathways (Cryan and Dinan, 2012; Dinan and Cryan, 2013). This emerging link between the gut microbiota and the central nervous system suggests that gut microbiota modification may have translational

applications in the treatment of neuropsychiatric disorders (Cryan and Dinan, 2015; Desbonnet et al., 2014; Hsiao et al., 2013).

Depression is a common, often recurrent (Eaton et al., 2008) heterogeneous disorder responsible for significant disability worldwide (WHO, 2008). The complex aetiology, involves dysregulated neuroendocrine (Stetler and Miller, 2011) neuroimmune (Dowlati et al., 2010), metabolic (Jokela et al., 2014) and neurotransmitter systems (Berton and Nestler, 2006). Current pharmacological interventions are suboptimal (Fava, 2003) and there has been little progress in the identification of biomarkers.

Data from animal studies provides evidence that the gut microbiota may impact on the neurobiological features of

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depression (Park et al., 2013), such as low-grade immune activation (Bailey et al., 2011), hypothalamic-pituitary-adrenal axis (HPA) activity (Sudo et al., 2004), altered tryptophan metabolism (Clarke et al., 2013; El Aidy et al., 2012; O'Mahony et al., 2015; Yano et al., 2015), neurotrophic factors (Bercik et al., 2011), and neurogenesis (Möhle et al., 2016; Ogbonnaya et al., 2015).

A number of studies have shown that when the microbiome is transplanted from one animal (either stressed or obese) to another control animal it can significantly alter anxiety-like behaviours, a common comorbidity of depression (Bercik et al., 2011; Bruce-Keller et al., 2015).

Different lactobacillus and bifidobacteria species have been shown to modulate depression and stress-related behaviours in animal models (Bravo et al., 2011; Desbonnet et al., 2010; Savignac et al., 2015). Furthermore, a growing number of small studies in healthy individuals suggest pre- and probiotic consumption can positively affect aspects of mood and anxiety (Messaoudi et al., 2011; Steenbergen et al., 2015), modulate HPA function (Messaoudi et al., 2011; Schmidt et al., 2015) and alter brain activity (Tillisch et al., 2013). However, there are a paucity of studies in relevant clinical populations (Jiang et al., 2015; Naseribafrouei et al., 2014; Zheng et al., 2016).

We investigated alterations in the gut microbiota composition in patients with depression with respect to signature physiological alterations in HPA axis function, immune activation and altered tryptophan metabolism. Next, we aimed to identify the functional consequences of the gut microbiota alterations in depression by determining levels of fecal short chain fatty acids. We then assessed gut permeability as a potential mechanism by which gut bacteria may influence brain function (Julio-Pieper et al., 2014; Kelly et al., 2015).

Finally, to confirm that an altered gut microbiota specifically influences aspects of depressive symptomatology, we carried out a fecal microbiota transplantation from depressed patients to a microbiota depleted antibiotic rat model and assessed if a depressive-like phenotype emerged in the treated animals.

1.1. Methods and materials

1.1.1. Subjects

Approval of the study protocol was granted by the Cork University Hospital (CUH) ethics committee and written informed consent was obtained from all subjects. The study was carried out in accordance with the Declaration of Helsinki. Thirty four depressed patients were recruited from outpatient and inpatient psychiatric clinics. Thirty three healthy subjects matched for gender, age and ethnicity were recruited (See [Supplementary Information](#)).

1.1.2. Microbiota & SCFA analysis

Extracted Fecal DNA was prepared for sequencing on the Illumina Miseq platform. The concentration of SCFA was measured using a Varian 3800 GC flame ionization system, fitted with a ZB-FFAP column (30 m × 0.32 mm × 0.25 mm; Sigma) (See [SI](#)).

1.1.3. Salivary cortisol analysis

Participants were instructed to collect three saliva samples using Salivettes (Sarstedt AG and Co, Numbrecht, Germany) at the following time points: (t0) upon waking, 30 min post waking (t+30), and 150 min post waking (t+150) (See [SI](#)).

1.1.4. Kynurenine/tryptophan determination

Plasma Tryptophan and kynurenine pathway metabolites were determined as previously described (Clarke et al., 2009). (See [SI](#)).

1.1.5. Cytokine determination

Plasma levels of IL-6, IL-8, TNF- α , and CRP were assayed in duplicate using high sensitivity commercially available electrochemiluminescence MULTI-SPOT[®] Meso Scale Discovery kits (MSD, Rockville, MD, 75USA) (See [SI](#)).

1.1.6. Lipopolysaccharide binding protein determination

LBP concentrations were determined using the Enzyme Immunoassay Kit for free human LBP (Enzo[®], Life Sciences).

1.1.7. Animals and treatments

All experiments were in full accordance with the European Community Council Directive (86/609/EEC). Adult male Sprague-Dawley rats (n = 28) were used and maintained as described in [SI](#). They were divided into control (n = 15) and depressed groups (n = 13) matched for average body weight. Rats were then given a cocktail of ampicillin and metronidazole (all at 1 g/L), vancomycin (500 mg/L), ciprofloxacin HCl (200 mg/L), imipenem (250 mg/L) once daily for 28 consecutive days in drinking water. Seventy-two hours later, animals were colonized via daily oral gavage of donor microbiota (300 μ L) for 3 days. Donor microbiota was acquired from pooled fecal samples from 3 of the most severely depressed male patients and 3 age and sex matched healthy controls. To offset potential confounder and/or cage effects and to reinforce the donor microbiota phenotype, booster inoculations were given twice per week throughout the study.

1.1.8. Behavioural testing

Sucrose preference; SP, Open field; OF, Elevated plus maze; EPM, Forced swim test FST were carried out as detailed in [Supplementary Materials](#).

1.1.9. Rat intestinal transit time determination

Rats were given 200 μ L of 6% carmin red in 0.5% methylcellulose (in PBS) given by oral gavage. The cages were inspected every 10 min post gavage and the appearance of the first red fecal pellet recorded.

1.1.10. Rat corticosterone determination

Plasma corticosterone levels were assayed using a commercially-available ELISA kit (Corticosterone EIA Kit, Enzo[®], Life Sciences).

1.1.11. Rat CRP and cytokine determination

Plasma CRP was determined using commercially available Ray-Bio[®] Rat CRP ELISA Kit. Cytokines were analyzed using a commercially available electrochemiluminescence multiplex system (MSD, Gaithersburg, MD, USA).

1.1.12. Rat lipopolysaccharide determination

Plasma LBP concentrations were determined using the Enzyme Immunoassay Kit (Enzo[®], Life Sciences).

1.1.13. Rat short chain fatty acid cecal content determination

The concentrations of SCFA were measured using a Varian 3800 GC flame-ionization system, fitted with a ZB-FFAP column (30 m × 0.32 mm × 0.25 μ m; Phenomenex, Macclesfield, Cheshire, UK).

1.1.14. Statistical analysis

Data that were normally distributed according to Shapiro-Wilk test were analyzed using unpaired t tests. Outliers were removed by Grubbs' test. Data that were not normally distributed were transformed by square root transformation. Microbiota data were analyzed using non parametric tests. Benjamini-Hochberg

procedure was used to correct for multiple comparisons with a FDR-adjusted p -value ≤ 0.1 considered significant. Statistical procedures were carried out using IBM SPSS 20.0. Graphs were generated using GraphPad Prism 5. Macronutrient data was generated using Diet Plan 6.

2. Results

2.1. Demographic data and health indicators

Other than education level, employment status, smoking and alcohol consumption, there were no differences between the groups (Table 1). Clinical characteristics of the depressed patients are presented in (Table 2).

2.2. Daily Macronutrient Consumption similar in depressed patients and controls

We assessed Daily Macronutrient Consumption using a food frequency questionnaire (Table S1). Apart from Trans fats ($t(61) = 2.06$, $p = 0.05$) there were no significant differences in diet between the groups.

2.3. Proinflammatory profile in depression

The data in this study confirm that the depressed group had increased levels of IL-6 ($t(62) = 2.69$, $p = 0.009$), IL-8 ($t(61) = 2.37$, $p = 0.021$), TNF- α , ($t(49) = 2.36$, $p = 0.022$) and CRP ($t(45) = 3.6$, $p = 0.001$) compared to the healthy controls (Fig. 1A–D).

2.4. Activated kynurenine pathway in depression

The kynurenine/tryptophan ratio was significantly higher in the depressed group compared to the controls ($t(61) = 2.01$, $p = 0.049$) (Fig. 1E). There were no significant differences in tryptophan ($t(58) = 0.92$, $p = 0.362$), kynurenine ($t(63) = 1.00$, $p = 0.320$), kynurenic acid ($t(45) = 0.38$, $p = 0.70$), or the kynurenic acid/kynurenine ratio ($t(44) = 0.40$, $p = 0.685$).

2.5. Altered HPA axis in depression

AUCg analysis showed that patients with depression exhibited a greater total cortisol output ($t(50) = 2.06$, $p = 0.05$) (Fig. 1F) but no difference in the delta cortisol response ($t(50) = -0.40$, $p = 0.69$) or AUCi ($t(50) = -0.67$, $p = 0.51$). There were no significant differences between baseline cortisol levels upon wakening ($t(50) = 1.88$, $p = 0.06$), 30 min post wakening ($t(50) = 1.28$, $p = 0.206$), or 150 min post wakening ($t(50) = 1.73$, $p = 0.09$) (Fig. 1G). Although there was no significant baseline difference between groups, when controlling for baseline cortisol values there was not a significantly elevated AUCg between groups.

2.6. No alterations in intestinal permeability in depression

There were no significant differences in plasma LBP levels ($t(63) = 1.05$, $p = 0.30$) between the groups.

2.7. Altered gut microbiota diversity & richness in depression

Chao1 richness ($U = 424$, $p = 0.005$), total observed species ($U = 441$, $p = 0.002$) and phylogenetic diversity ($U = 447.5$, $p = 0.001$) were decreased in the depressed group. There was no difference in Shannon diversity ($U = 350$, $p = 0.197$) (Fig. 2A–D). Significant differences in beta diversity between the healthy and depressed groups (Bray-Curtis ($p = 0.014$), unweighted unifrac ($p = 0.002$) and weighted unifrac ($p = 0.018$) were unable to separate groups according to PCoA analysis (Figure S3A–C). The difference of the global microbiota composition from the 16S rRNA data of the depressed and control groups was assessed by ordination. Statistics based on random permutations of the redundancy analysis (RDA) showed that the depressed group is significantly separated at genus level ($p = 0.03$) from the control group (Fig. 2E).

2.8. Altered gut microbiota composition in depression

At the phylum level, there were no statistically significant differences in the relative abundances between the depressed group and the healthy controls (data not shown). At the family level, the

Table 1

Comparison of group demographics and health indicators. Data are presented as mean and S.D, or median and range. BMI, body mass index; HTN, Hypertension; IPAQ, International Physical Activity Questionnaire. * $p < 0.05$ vs control. ** $p < 0.01$ vs control; *** $p < 0.001$ vs control.

	Healthy controls (n = 33)	Depression (n = 34)	p-value
Age mean (s.d.)	45.8 (11.9)	45.8 (11.5)	0.98
Sex:			
Male (%)	19 (57.6)	21 (61.8)	0.73
Female (%)	14 (42.4)	13 (32.8)	
Education, degree level (%)	26 (78.8)	8 (23.5)	<0.001***
Relationship status (% yes)	23 (69.7)	20 (58.8)	0.35
Employed (% yes)	31 (93.9)	16 (47.1)	<0.001***
Alcohol units/week mean, (s.d)	9.24 (8.3)	5.29 (5.9)	0.03*
1 st degree relative with alcohol use disorder	4 (12.1)	12 (35.3)	0.03*
Smoking (%)			
Current	3 (9.1)	13 (38.2)	
Ex	19 (57.6)	3 (8.8)	
Never	11 (33.3)	18 (52.9)	<0.001***
BMI mean (s.d.)	24.58 (2.7)	26.2 (4.5)	0.07
Dyslipidaemia (%)	4 (12.1)	7 (20.6)	0.51
HTN (%)	3 (9.1)	3 (8.8)	0.97
Physical Activity (IPAQ)			
Low (%)	7 (21.2)	13 (38.2)	
Moderate (%)	16 (48.5)	14 (41.2)	
High (%)	10 (30.3)	6 (17.6)	0.23
Metabolic Equivalent Task Units (MET) median, range	1386 (7287)	693 (7722)	0.10
Hours sitting per day mean, (s.d)	6.03 (2.730)	5.97 (2.456)	0.92

Table 2
Comparison of group clinical characteristics. Data are presented as mean and S.D, or median and range. * $p < 0.05$ vs control. ** $p < 0.01$ vs control; *** $p < 0.001$ vs control.

	Healthy controls (n = 33)	Depression (n = 34)	p- value
Diagnosis			
Depression (%)	NA	29 (85.3)	
BPAD II (%)	NA	5 (14.7)	
Co-morbid Anxiety Disorder (%)	NA	4 (11.7)	
Ex-Alcohol abuse (%)	NA	8 (23.5)	
Ex-Substance abuse (%)	NA	3 (8.8)	
Ex-Alcohol & Substance abuse (%)	NA	2 (5.8)	
HAMD 17 median (range)	NA	19.5 (14)	
Beck Depression mean, (s.d)	NA	32.4 (9.92)	
Duration of Depressive sx (months) median, (range)	NA	3.0 (72)	
Number of Depressive episodes median (range)	NA	1.0 (8)	
Positive Family History of Depression (%)	2 (6.1)	21 (61.8)	<0.001***
Beck Anxiety median, (range)	NA	25.5 (45)	
Perceived Stress Scale (PSS) mean, (s.d)	7.5 (4.9)	27.7 (6.0)	<0.001***
Pittsburgh Sleep Quality Index (PSQI) mean, (s.d)	2.8 (1.8)	11.7 (4.3)	<0.001***

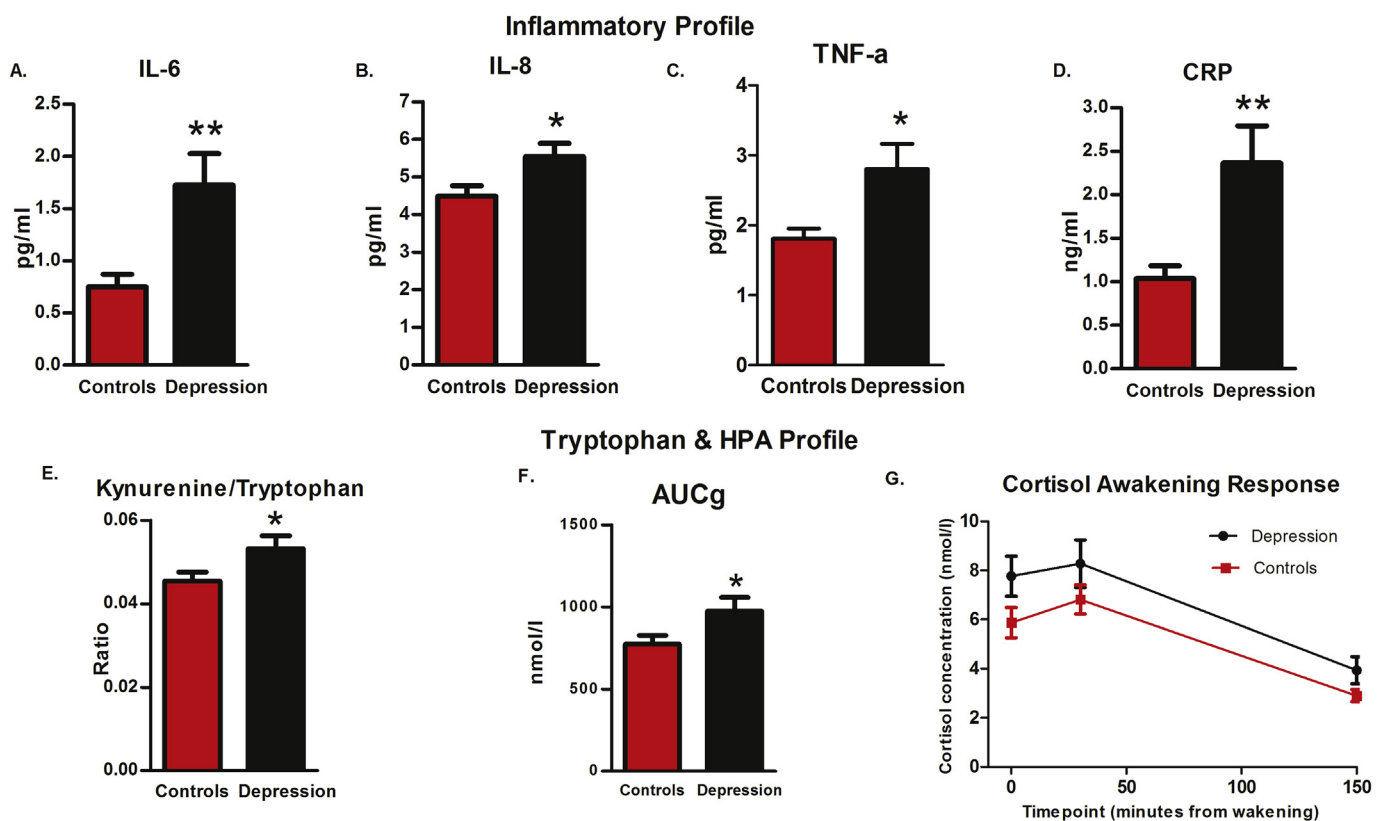


Fig. 1. Altered Inflammatory, Tryptophan, and HPA profile in depressed patients. The depressed patients had significantly increased levels of (A) IL-6, ($p = 0.009$), (B) IL-8, ($p = 0.021$) (C) TNF- α ($p = 0.022$) and (D) CRP ($p = 0.001$) compared to the healthy controls. The depressed group had a significantly increased (E) Kynurenine/tryptophan ratio ($p = 0.049$) and a greater cortisol output as measured by the (F) Area under the Curve with respect to ground (AUCg) ($p = 0.045$), though no significant difference in the (G) Cortisol Awakening Response (CAR) ($p = 0.21$).

relative proportions of *Prevotellaceae* ($U = 355$, $p = 0.007$) were decreased, whereas *Thermoanaerobacteriaceae* were increased in the depressed group ($U = 52.5$, $p = 0.021$) (Fig. 2F). At the genus level, the relative proportions of *Eggerthella* ($U = 21.0$, $p = 0.009$), *Holdemania* ($U = 146.5$, $p = 0.023$), *Gelria* ($U = 52.5$, $p = 0.021$), *Turicibacter* ($U = 89$, $p = 0.034$), *Paraprevotella* ($U = 119$, $p = 0.041$), and *Anaerofilum* ($U = 50.5$, $p = 0.021$) were increased in the depressed group, whereas *Prevotella* ($U = 324.5$, $p = 0.022$) and *Dialister* ($U = 153.5$, $p = 0.032$) were decreased (Fig. 2G).

2.9. Short chain fatty acids

There were no significant differences between groups in the fecal SCFAs, acetate ($t(49) = 0.457$, $p = 0.65$), propionate ($t(49) = 0.103$, $p = 0.918$), iso-Butyrate ($t(43) = 0.678$, $p = 0.501$) or butyrate ($t(49) = -0.168$, $p = 0.867$) (Fig. 3A).

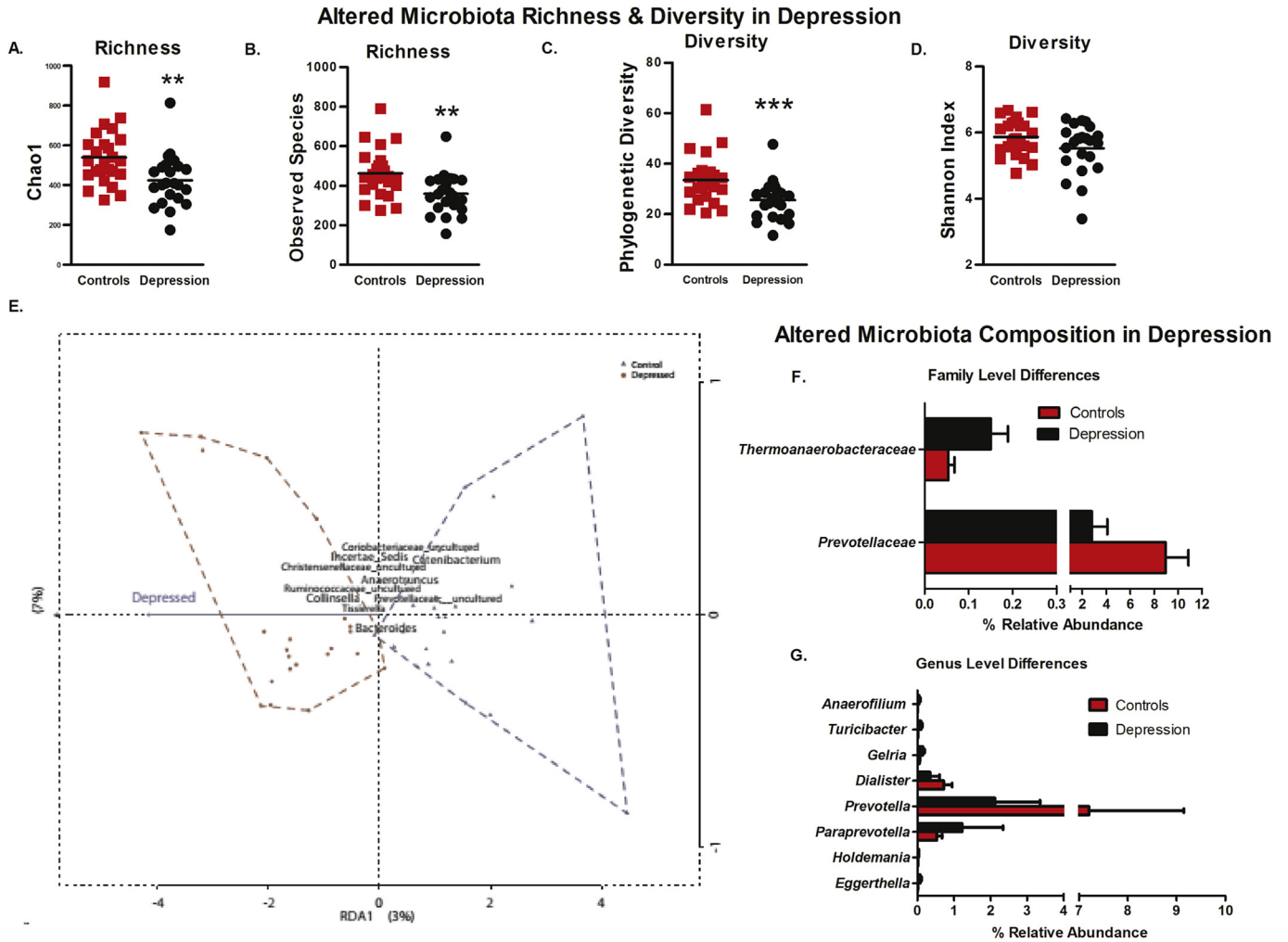


Fig. 2. Altered Gut Microbiota Composition, Diversity & Richness in depressed patients. There was a significant reduction in richness as measured by (A) Chao1, ($p = 0.005$) and (B) Total observed species, ($p = 0.002$). The depressed group showed reduced (C) phylogenetic Diversity, ($p = 0.001$), but no significant differences in (D) Shannon diversity, ($p = 0.197$). (E) The significant differences at genus level between the depressed group and the controls which cluster by group in a Redundancy analysis (RDA) plot ($p = 0.03$). (F) Significant Family level differences and (G) significant genus level differences between depressed patients and healthy controls in % relative abundances.

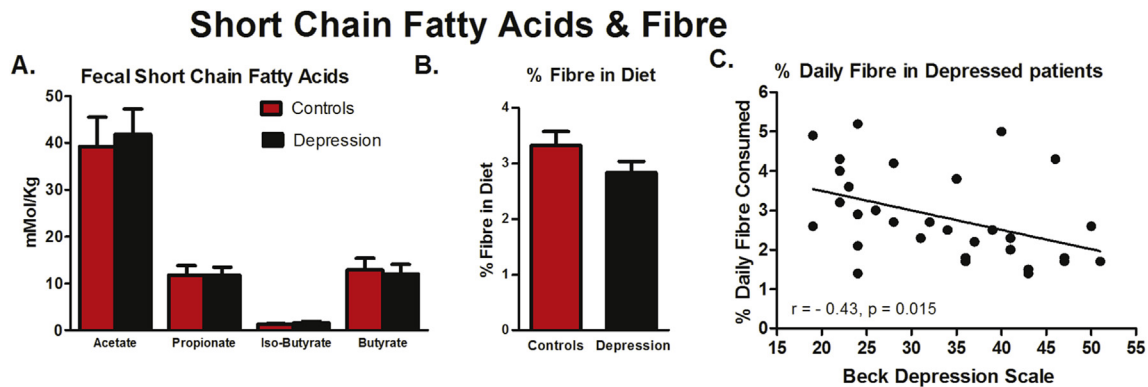


Fig. 3. Functional Implications of an altered Gut Microbiota. (A) There were no significant differences in fecal SCFAs. (B) There were no significant differences in the daily % fibre consumed between the groups ($p = 0.134$) (C) though within the depressed group the daily % fibre negatively correlated with the Beck Depression Scale ($r = -0.43$, $n = 31$, $p = 0.015$).

2.10. No alterations in fecal metabolites

A Principal Component Analysis (PCA) model did not reveal

distinct grouping of the depression samples compared to the healthy control samples (Fig. S2).

2.11. Adoptive transfer of depressive phenotype

2.11.1. Behaviour

Rats that received the FMT from the depressed pool demonstrated anhedonia-like behaviours as assessed in the sucrose preference test ($t(12) = 2.628$, $p = 0.022$) (Fig. 4B) with a significant decrease in sucrose intake without affecting fluid intake. Rats receiving FMT from depressed patients also exhibited anxiety-like behaviours as demonstrated by a significant decrease in visits to the open arms ($t(12) = 2.471$, $p = 0.029$) in the elevated plus maze (Fig. 4C) and a reduction in time spent in the centre in the open field ($t(24) = 2.662$, $p = 0.013$) (Fig. 4D). These changes occurred without alterations in overall activity as measured in the total visits to the closed and open arms in the EPM and total activity in the open field. In the forced swim test, there were no significant differences between the groups in immobility time ($t(26) = -0.43$, $p = 0.966$), swimming ($t(26) = 1.164$, $p = 0.255$) or climbing ($t(26) = 0.629$, $p = 0.535$) (Fig. 4E).

2.11.2. Neurobiology of depression emerges following FMT

Rats that received the depression FMT had significantly increased plasma kynurenine levels ($t(25) = 2.3$, $p = 0.029$) and an increased plasma kynurenine/tryptophan ratio ($t(25) = 2.9$

$p = 0.008$) (Fig. 4F) but no differences in plasma tryptophan levels ($t(25) = 0.41$, $p = 0.686$). There was a trend toward increased levels of plasma CRP in rats that received the depression FMT ($t(25) = 1.803$, $p = 0.083$) (Fig. 4G) though no differences in the levels of plasma IL-6, ($t(23) = -0.173$, $p = 0.864$), TNF- α , ($t(24) = 0.569$, $p = 0.574$) or IL1b ($t(24) = 1.716$, $p = 0.09$). There were no significant differences in plasma LBP levels ($t(22) = 0.15$, $p = 0.878$) (Fig. 4H) or plasma corticosterone levels ($t(26) = 0.063$, $p = 0.949$) (Fig. 4I). There were also no significant differences in hippocampal Bdnf expression ($t(26) = 0.312$, $p = 0.757$) (Fig. S4).

2.11.3. Gastrointestinal physiology altered following FMT

Rats receiving the depression FMT demonstrated a significant increase in intestinal transit time ($t(26) = 2.652$, $p = 0.013$) (Fig. 4J).

2.11.4. Altered Gut Microbiota Richness, diversity, composition & SCFAs following FMT

Rats that received the FMT from depressed patients had reduced gut microbiota richness as measured by Chao1 ($p = 0.004$) and observed species ($p = 0.006$) and reduced diversity measured by phylogenetic diversity ($p = 0.006$) and Shannon index ($p = 0.002$) (Fig. 5A–D).

At the phylum level, the relative abundances of *Actinobacteria*

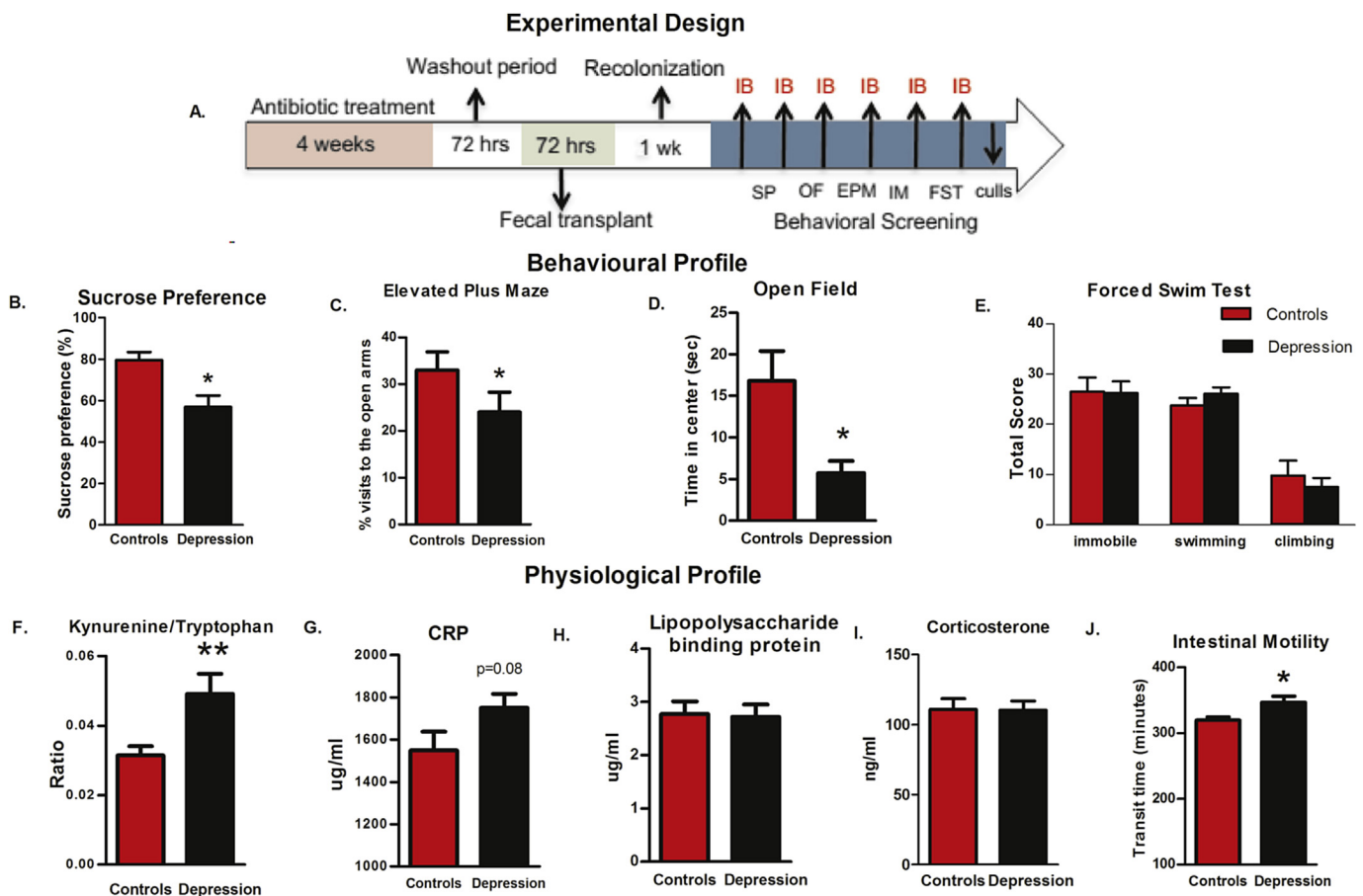
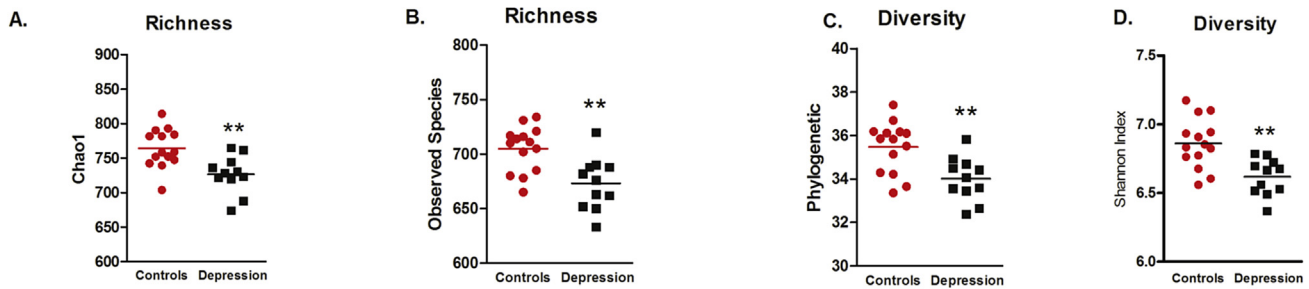


Fig. 4. Behavioural and physiological changes following fecal transplantation. (A) Experimental design (Sucrose preference; SP, Open field; OF, Elevated plus maze; EPM, Intestinal motility; IM, Forced swim test FST, Inoculation boost; IB (twice a week). The rats that received the depression FMT exhibited (B) anhedonia like behaviour, indicated by a decrease in the 24 h 1% sucrose preference test ($p = 0.022$) and an increase in anxiety like behaviour measured by (C) a decrease in open arm visits in the elevated plus maze (EPM) ($p = 0.029$) and (D) a decrease in time spent in the open field ($p = 0.013$) and compared to rats that received the healthy FMT. (E) There were no differences in the immobility, swimming or climbing time in the Forced Swim Test (FST). (F) There was an increase in the plasma Kynurenine/Tryptophan ratio ($p = 0.008$) in the rats that received the FMT from depressed patients. (G) There was a trend for increased plasma CRP levels in the rats that received the FMT from depressed patients ($p = 0.083$). There were no significant differences in (H) plasma Lipopolysaccharide binding protein (LBP) levels or (I) plasma corticosterone levels 15 min post-acute FST stressor. (J) Rats that received the FMT from depressed patients demonstrated a significant increase in intestinal transit time ($p = 0.013$).

Altered Gut Microbiota Richness & Diversity following fecal transplantation



Altered Gut Microbiota Composition & SCFAs following fecal transplantation

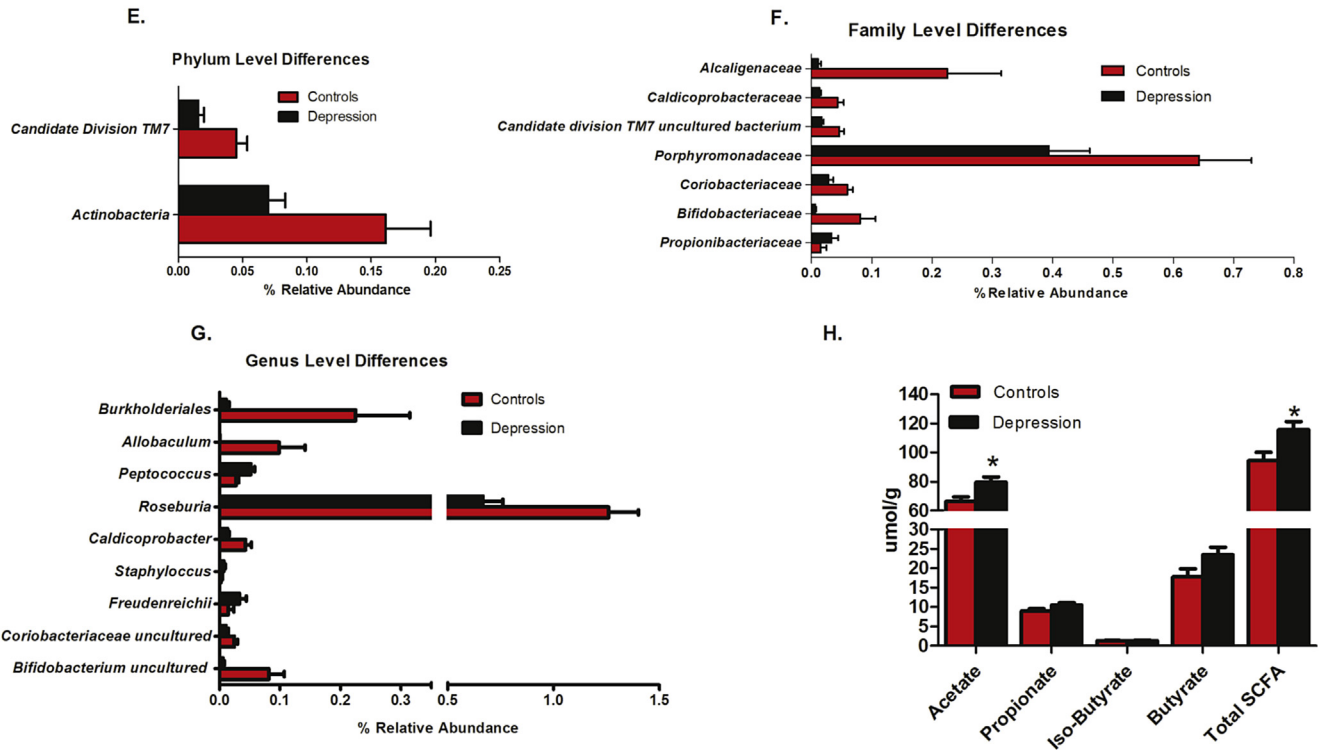


Fig. 5. Altered Gut Microbiota Richness, Diversity & SCFAs following fecal transplantation. Rats that received the FMT from depressed patients had reduced gut microbiota richness as measured by (A) Chao1 ($p = 0.004$) and (B) observed species ($p = 0.006$) and reduced diversity measured by (C) phylogenetic diversity ($p = 0.006$) and (D) Shannon index ($p = 0.002$). Significant differences at the phylum level (E), the family level (F) and the (G) genus level between the rats that received the FMT from the depressed patients compared to the rats that received the healthy FMT. (H) Levels of fecal acetate and total SCFAs were increased in the rats that received the depression FMT ($p = 0.011$). There was a trend toward significant increases in the levels of propionate ($p = 0.068$) and butyrate ($p = 0.06$) following FMT from depressed patients.

($U = 32$, $p = 0.013$) and *Candidate Division TM7* ($U = 28.5$, $p = 0.006$) were decreased in rats that received the depression FMT (Fig. 5E). At the family level, the relative proportions of *Bifidobacteriaceae* ($U = 138.5$, $p = 0.001$), *Coriobacteriaceae* ($U = 129$, $p = 0.004$), *Porphyromonadaceae* ($U = 123$, $p = 0.012$), *Candidate division TM7 uncultured bacterium* ($U = 128$, $p = 0.005$), *Caldicoprobacteraceae* ($U = 126.5$, $p = 0.007$), *Alcaligenaceae* ($U = 144$, $p < 0.000$) were decreased in rats that received the depression FMT. *Propionibacteriaceae* ($U = 27$, $p = 0.006$) was increased in the rats that received the depression FMT (Fig. 5F).

At the genus level, the relative abundances of *Bifidobacterium uncultured* ($U = 136$, $p = 0.001$), *Coriobacteriaceae uncultured* ($U = 128$, $p = 0.005$), *Caldicoprobacter* ($U = 124$, $p = 0.01$), *Roseburia* ($U = 132$, $p = 0.003$), *Allobaculum* ($U = 126$, $p = 0.004$), *Burkholderiales* ($U = 146$, $p < 0.000$) were decreased in rats that received the depression FMT. *Freudenreichii* ($U = 26$, $p = 0.004$), *Staphylococcus* ($U = 37$, $p = 0.013$), *Peptococcus* ($U = 27.5$, $p = 0.006$)

were increased in rats that received the depression transplantation (Fig. 5G).

Fecal acetate and total SCFAs were increased in the rats that received the depression FMT ($p = 0.011$). There was a trend toward significant increases in the levels of propionate ($p = 0.068$) and butyrate ($p = 0.06$) following FMT from depressed patients (Fig. 5H).

3. Discussion

The present findings represent definitive evidence that depression-associated alterations in the gut microbiome are sufficient to disrupt behavioural and physiological homeostasis. Specifically, transplantation of the perturbed microbiota signature from depressed patients to microbiota-depleted rats induced the development of some of the behavioural and physiological features of the depressive phenotype. Furthermore, this data indicates that a

gut microbiota transfer from depressed patients could serve as a novel animal model of depression in the context of microbiome-gut-brain axis dysfunction.

Depression is associated with an altered gut microbiota composition, richness and diversity. It was possible to differentiate the depressed group from the healthy control group based on differences at the genus level using redundancy plot analysis (Fig. 2E). A major parallel between the depressed patient profiles and the rats that received the FMT from the depressed patients encompassed a reduction in richness and alpha diversity (Fig. 5A–D). The most pronounced difference was observed in the reduction of *Prevotellaceae* family and subsequently in the *Prevotella* genus, previously found to be decreased in Parkinson's disease patients (Gustafsson et al., 2015; Scheperjans et al., 2015). A significant association has been noted between the Mediterranean diet, regarded as a preventive strategy in depression, and *Prevotella* (De Filippis et al., 2015; Opie et al., 2015). However, there were no significant differences in *Prevotellaceae* or *Prevotella* in the rats that received the depression FMT.

Given, the fact that the rodent and human microbiota are different (Nguyen et al., 2015) and the inherent translational challenges in moving from rodent to man, it is perhaps not surprising that overlap of specific taxa are not reflected in the rat microbiota following the FMT. In addition, the microbiota composition was determined using fecal samples in the human study and from cecal samples in the rodent study. Considering, not all behavioural readouts of relevance to depression were recapitulated in our preclinical study, this suggests that those domains which were impacted relate to the missing taxa which are a feature of the rodent microbiota following the transfer.

We have demonstrated, that transferring the gut microbiota from depressed patients to rats with a depleted gut microbiota can induce the development of some of the features of the depressive phenotype, such as anhedonia and anxiety-like behaviours, and produce a physiological profile similar to depressed individuals. In contrast to a recent FMT study from depressed patients using germ-free mice (Zheng et al., 2016), we did not observe alterations in the FST. While GF animals are an excellent proof-of-principal tool, we and others have shown that these animals have some profound CNS abnormalities in adulthood as a consequence of GF status during critical neurodevelopmental windows, many of which are relevant for the depressive phenotype. The main advantage then of antibiotic-induced microbiota depletion during adulthood is that it avoids these potential confounding influences (Arrieta et al., 2016; Luczynski et al., 2016). Our data is consistent with the view that the emergence of only some of the behavioural and neurobiological correlates of depression are contingent on the gut microbiota (De Palma et al., 2015). Future studies will need to address the precise temporal dynamics of the emergence and possible persistence of the behavioural alterations.

We show that depression is associated with dysregulated tryptophan metabolism as indicated by an increased plasma kynurenine/tryptophan ratio. Rats that received the depression FMT also had an increased kynurenine/tryptophan ratio. Together with the potential impact on serotonin, increased microbiota-mediated degradation of tryptophan along this pathway has a broad range of implications for multiple neurotransmitter systems (Schwarcz et al., 2012). The rate of tryptophan metabolism along the kynurenine pathway is dependent on the activity of indoleamine-2,3-dioxygenase (IDO), an enzyme induced by cytokines, and tryptophan-2,3-dioxygenase (TDO), the expression of which can be induced by circulating glucocorticoids (O'Connor et al., 2009) and has been reported to be regulated by the gut microbiota during colonization (El Aidy et al., 2014).

The pro-inflammatory profile was also partially transferred,

with a trend towards an increase in plasma CRP albeit in the absence of alterations in plasma IL-6, TNF- α , or IL1b, in the rats that received the depressed FMT. At the fecal metabolomic level there were no significant differences between depressed patients and healthy controls (Fig. S2). Taken together, these findings, in conjunction with an increased kynurenine/tryptophan ratio (a proxy marker for an activated immune system), suggests that an immune mediated mechanism may account for the development of the depressive phenotype in the rats. A dysregulated HPA axis function was not observed following the FMT, at least in terms of corticosterone output following an acute stressor. However, single time point analysis of HPA axis function can be unreliable (Allen et al., 2014). Rats that received the depression FMT demonstrated increased intestinal transit time, suggestive of colonic motility dysfunction. Altered gastro-intestinal function is a well-established but often neglected characteristic of depression (Gorard et al., 1996; Haug et al., 2002).

A significant increase in the order *Bacteroidales* and a decrease in *Lachnospiraceae* family compared to controls was previously shown in depressed patients (Naseribafrouei et al., 2014). Another study separated the depressed patients into an actively depressed group and those that had responded to treatment (Jiang et al., 2015). Similar to our study, they demonstrated a decrease in *Prevotellaceae* and *Prevotella* in depressed patients. They reported no differences in circulating pro-inflammatory cytokines in contrast to low grade inflammation described in depressed patients (Dinan, 2009). Dietary factors, geography or methodological differences during sampling, processing or analysis may also account for discrepancies (Kelly et al., 2016).

The impact of stress on the gut microbiome might also be a factor and we have previously shown that early life stress can remodel the gut microbiota (O'Mahony et al., 2009). Subtle alterations in microbiota acquisition or maintenance during this vulnerable early life period may act to impact on (neuro)endocrine and (neuro)immune signalling pathways of the brain-gut-microbiota axis, disruption of which may subsequently predispose to stress-related disorders in adulthood (Sudo et al., 2004). The depressed group in our study did experience more stressful life events prior to the age of 17 years and in the last 3 years compared to the healthy group (Figure S1A).

Studies have demonstrated higher IgA- and IgM-mediated immune responses directed against LPS of certain commensal gram negative gut bacteria in depression (Maes et al., 2012, 2008, 2013). Bacterial DNA has been detected in whole serum from depressed patients who also displayed increased TLR4 expression on peripheral mononuclear blood cells compared to healthy controls (Keri et al., 2014). Although we demonstrate a proinflammatory profile in the depressed group, we found no statistically significant difference in intestinal permeability, as measured by LBP. We cannot rule out the possibility of glucocorticoid receptor resistance playing a role in the dysregulated HPA axis and inflammatory function in the depressed patients (Calfa et al., 2003; Pariante and Miller, 2001).

Although the percentage of daily fibre consumed was the same between our depressed and healthy groups, we found a negative correlation with depressive symptoms in our depressed group suggesting an important role for fibre, and fermentation products of fibre in depression (Fig. 3C). Microbial metabolites such as SCFAs can reach the circulation, cross the blood brain barrier (Frost et al., 2014; Vijay and Morris, 2014) and activate specific receptors in relevant brain regions underpinning the neurocircuitry pertinent to the expression of depression and anxiety-related behaviours (Schroeder et al., 2007; Wei et al., 2015). Our preclinical data does show that a depression-associated microbiota can impact SCFA production (Fig. 5H), however, our clinical data does not show

alterations in SCFAs or in the global metabolite profile (Fig. S2). Although the gut microbiota can impact transcriptional regulation (Stilling et al., 2015) we found no significant differences in hippocampal Bdnf expression in the rats that received the FMT.

3.1. Limitations

The majority of depressed patients in this study were prescribed antidepressant medication (Fig. S1). We acknowledge this as a potential confounding factor and the possibility of an antidepressant-related and serotonin-driven contribution to the alteration in the gut microbiota. However, antidepressants would be expected to reduce the kynurenine/tryptophan ratio rather than increase it via, for example, activity on TDO (Badawy and Morgan, 1991; Badawy et al., 1991) making it unlikely that residual medication in the fecal transplantation from the depressed patients would increase depressive and anxiety like behaviours in the rats that received the depressed FMT. Regardless of the origins of the gut microbiota differences in the transplant, the preclinical data confirms that when a depression-associated microbiota is transferred, neurobiological and behavioural consequences can ensue.

4. Conclusions

We show that depression is characterised by alterations in the gut microbiota. We have demonstrated that it is possible to reproduce aspects of depressed behaviour and physiology via a gut microbiota transfer. This suggests that the gut microbiota could play a causal role in the complex mechanisms underlying the development of depression. The profile of depression-like behaviours and physiological alterations noted following FMT suggests that this represents a novel paradigm in behavioural pharmacology to investigate microbiota-associated depression. Findings from this study advance the concept that targeting the gut microbiota may be a viable therapeutic strategy for novel antidepressant development in sub groups of depressed patients and may augment depression prevention strategies.

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Contributors

Dr Kelly and Dr Borre contributed to study design, data processing, data collection, data analysis, and manuscript preparation. Ciaran O' Brien, Dr Patterson, Dr El Aidy, Dr Kennedy, Jennifer Deane, Sasja Beers, Dr Karen Scott, Dr Gerard Moloney and Alan E. Hoban contributed to data processing and data analysis. Dr Lucinda Scott and Patrick Fitzgerald contributed to data collection. Prof Ross, Prof Stanton, Dr Clarke, Prof Cryan and Prof Dinan contributed to study design, data analysis, and manuscript preparation. All authors have approved the final manuscript.

Declaration of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jpsychores.2016.07.019>.

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