Towards an understanding of Depression-Associated Gut Microbiota Induced Neurobehavioural Changes in the Rat

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Introduction

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 [1][2]. The pathophysiology of depression involves neuroimmune-neuroendocrine dysregulation [3][4]. Depression is associated with decreased gut microbiota richness and diversity [5]. However, the underlying mechanisms by which changes in the gut microbiota composition and function contribute to the pathophysiology of depression have yet to be fully elucidated [6].

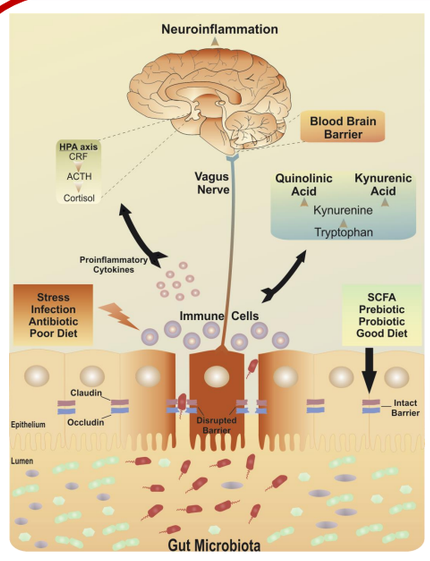


Figure 1: Signaling pathways involved in The Brain Gut Microbiota Axis [4].

Aims

1. To determine the composition, richness and diversity of the gut microbiota in Depressed patients compared to healthy control participants and its relationship to: Short Chain Fatty acids (SCFAs), immune activity (plasma cytokines), Hypothalamic-Pituitary-Adrenal axis (HPA-axis) function and Tryptophan metabolism

2. To determine the behavioral & physiological effects of a Fecal Microbiota Transplantation from Depressed patients & health controls to a microbiota depleted antibiotic rat model

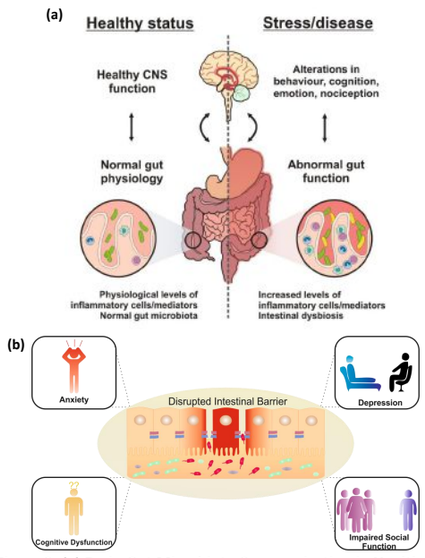


Figure 2: (a) Brain Gut Microbiota Communication in Health & Disease [7]. **(b) Activation of brain-gut-microbiota Axis signalling pathways via a compromised intestinal barrier with potential effects on mood, anxiety, cognition and social interaction** [4].

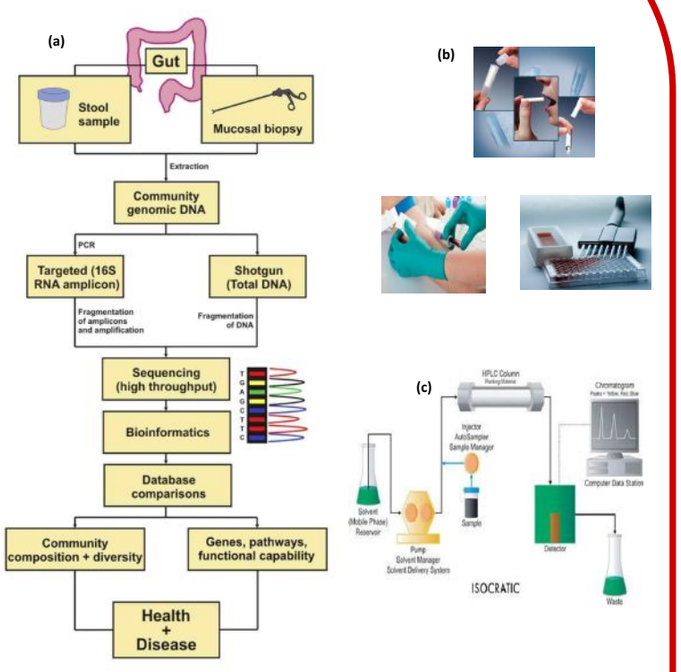
Methods

Study Population:
34 patients with DSM IV Major Depressive Disorder
33 healthy subjects matched for sex, age, BMI & Ethnicity (see sample characteristics)

Measures:
Gut Microbiota Structure & Diversity:
16s rRNA gene sequencing
Microbial Metabolites: Metabolomics,
Short chain fatty acids (Gas Chromatography)
Hypothalamic-Pituitary-Adrenal (HPA) Axis:
Salivary Cortisol (ELISA)
Inflammatory:
Plasma cytokines & CRP (Meso Scale Discovery)
Tryptophan Metabolites:
Plasma tryptophan & kynurenine (HPLC)
Intestinal Permeability:
Plasma Lipopolysaccharide Binding Protein
Subjective Mood & Stress:
Hamilton Depression rating scale (HAMD 17)
Beck Depression & Anxiety scales (BDI & BAI)
Perceived Stress scale (PSS)
Pittsburgh Sleep Quality Index (PSQI)
Diet:
Food Frequency Questionnaire (FFQ)
Exercise:
International Physical Activity Questionnaire (IPAQ)

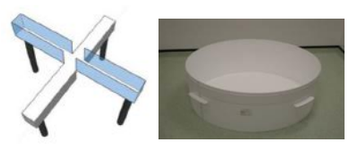
Rats:
28 Male Sprague-Dawley rats
Behavioural tests: Sucrose preference (SP), Open field (OF), Elevated plus maze (EPM), Intestinal motility (IM), Forced swim test (FST), Inoculation boost (IB) (twice a week)

Physiological outputs:
HPA Axis: Corticosterone 15 mins post FST
Inflammatory: plasma cytokines & CRP
Tryptophan Metabolites: plasma tryptophan & kynurenine (HPLC)
Intestinal Permeability: plasma Lipopolysaccharide Binding Protein
Intestinal Motility: Transit Time
Hippocampal Bdnf Gene Expression: Quantitative real-time PCR (qRT-PCR)



(d)

Antibiotic cocktail	Drinking water
Ampicillin	1 g/L
Vancomycin	500 mg/L
Ciprofloxacin HCl	200 mg/L
Imipenem	250 mg/L
Metronidazole	1 g/L



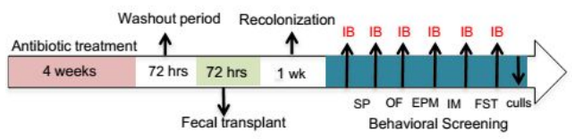


Figure 3: **(a)** Procedural stages of gut microbiota sampling & sequencing, **(b)** biomarker collection and analysis, **(c)** procedural stages of HPLC, **(d)** experimental design .

Sample Characteristics

Demographics & Health Measures	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
BMI mean (s.d.)	24.58 (2.7)	26.2 (4.5)	0.07
HAMD 17 median (range)	NA	19.5 (14)	NA
Beck Depression mean, (s.d)	NA	32.4 (9.92)	NA
Beck Anxiety median, (range)	NA	25.5 (45)	NA

Neurobiology of Depression

Elevated Inflammatory Markers, Altered Tryptophan & HPA Axis Activity in Depression

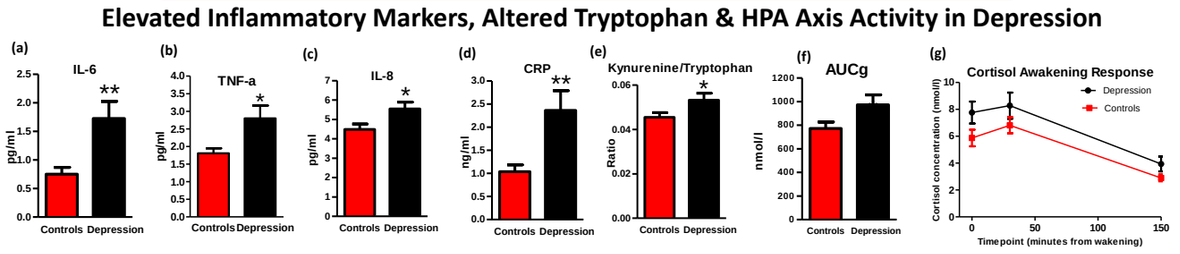


Figure 4: Depressed patients had significantly increased levels of **(a)** IL-6, (p = 0.009) **(b)** TNF-α (p = 0.022), **(c)** IL-8 (p = 0.021) and **(d)** CRP, (p = 0.001) compared to the healthy controls and had a significantly higher **(e)** kynurenine/tryptophan ratio (p = 0.049), and had an increased cortisol output indicated by **(f)** Area under the Curve with respect to ground (AUCg) (p = 0.045) and the **(g)** Cortisol Awakening Response (CAR) (p=0.026)

Altered Gut Microbiota Composition & Reduced Diversity & Richness in Depression

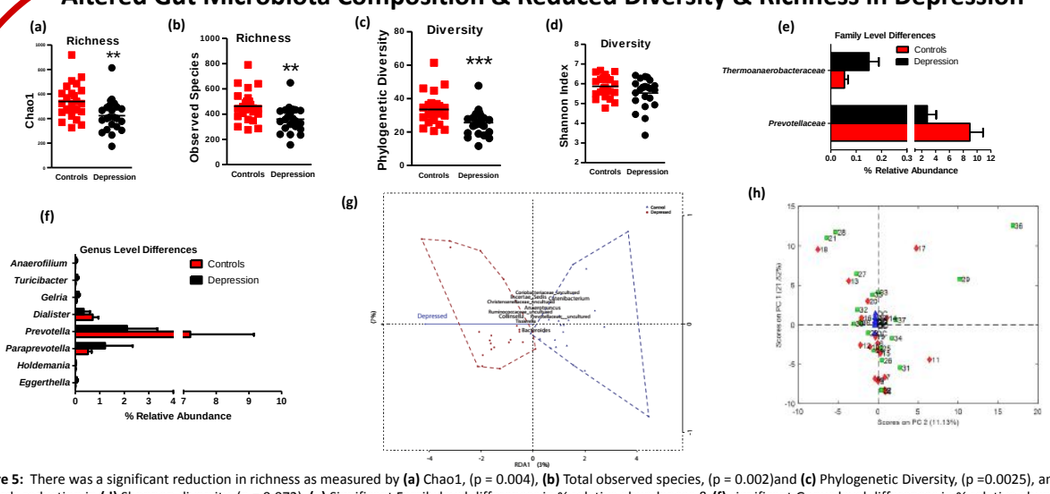


Figure 5: There was a significant reduction in richness as measured by **(a)** Chao1, (p = 0.004), **(b)** Total observed species, (p = 0.002)and **(c)** Phylogenetic Diversity, (p =0.0025), and a trend towards reduction in **(d)** Shannon diversity, (p = 0.072). **(e)** Significant Family level differences in % relative abundances & **(f)** significant Genus level differences in % relative abundances. **(g)** The significant differences at genus level between the depressed group and the controls which cluster by group in a Redundancy analysis plot. There were no distinct groupings in Fecal Metabolites in a **(h)** a score plot from a Principal Component Analysis (PCA) model calculated on the relative concentrations of the in the variables (Red represents depressed patients and green represents healthy controls, blue triangles represents a mixed pooled sample (QC sample).

Fecal Microbiota Transfer from Depressed patients Induces alterations in Gut Microbiota Composition, Richness, Diversity & SCFAs in Rats

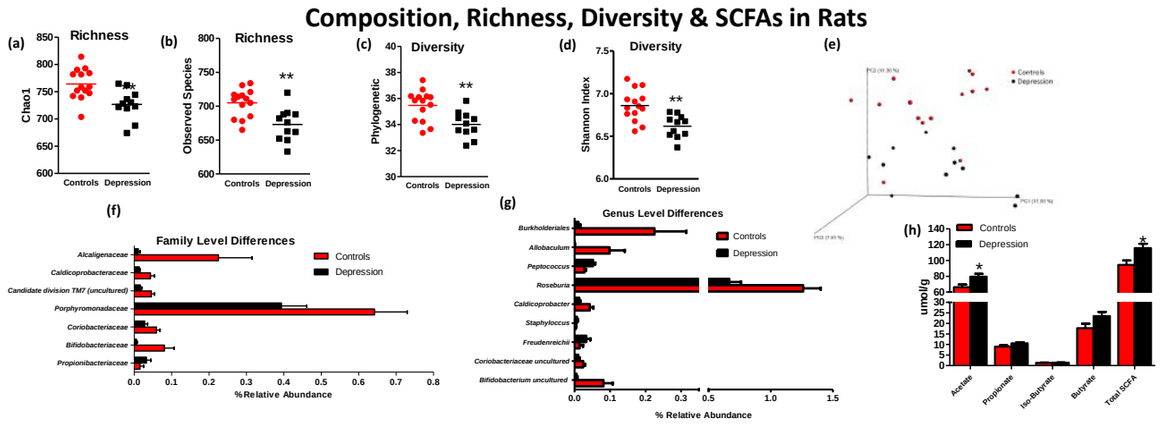


Figure 6: Rats that received the FMT from depressed patients had reduced gut microbiota richness as measured by **(a)** Chao1 (p = 0.004) and **(b)** observed species (p = 0.006) and reduced diversity measured by **(c)** phylogenetic diversity (p = 0.006) and **(d)** Shannon index (p = 0.002). **(e)** PCoA plot representing unweighted unifrac beta diversity in the rats. Significant differences at the **(f)** family level 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.

Fecal Microbiota Transfer from Depressed patients Induces Depressive like behaviour & physiology in Rats

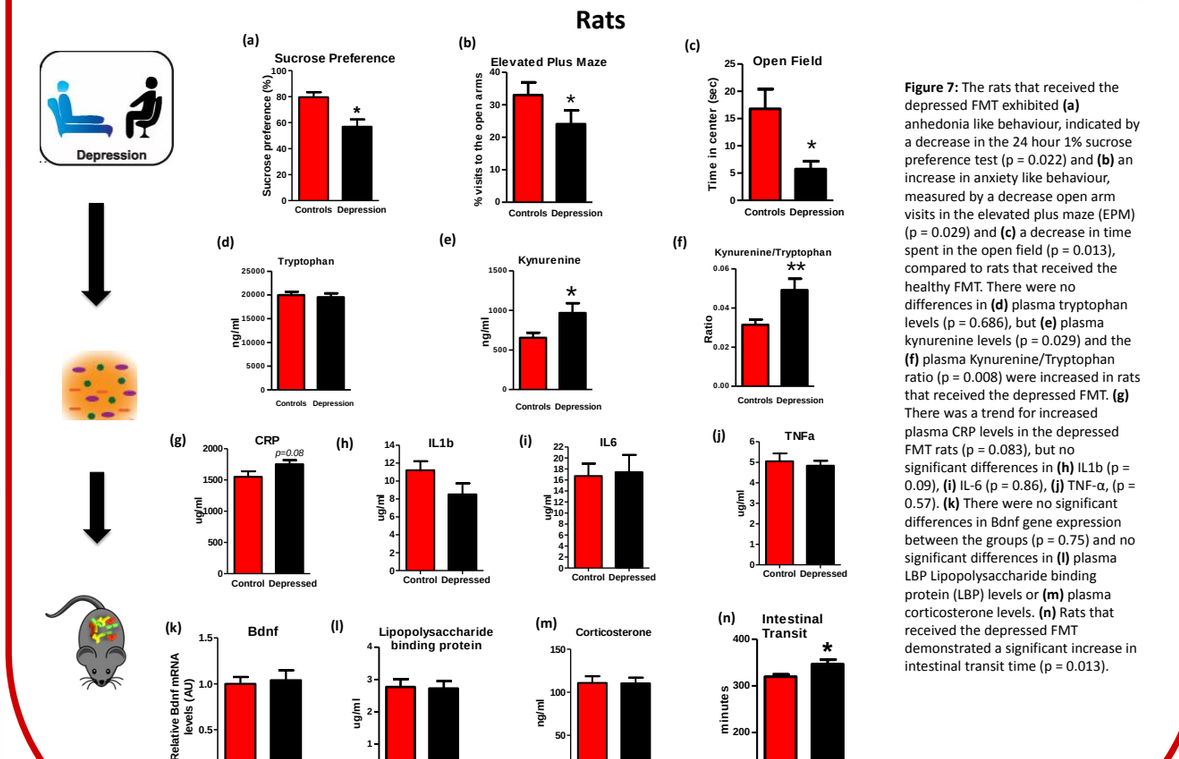


Figure 7: The rats that received the depressed FMT exhibited **(a)** anhedonia like behaviour, indicated by a decrease in the 24 hour 1% sucrose preference test (p = 0.022) and **(b)** an increase in anxiety like behaviour, measured by a decrease open arm visits in the elevated plus maze (EPM) (p = 0.029) and **(c)** a decrease in time spent in the open field (p = 0.013), compared to rats that received the healthy FMT. There were no differences in **(d)** plasma tryptophan levels (p = 0.686), but **(e)** plasma kynurenine levels (p = 0.029) and the **(f)** plasma Kynurenine/Tryptophan ratio (p = 0.008) were increased in rats that received the depressed FMT. **(g)** There was a trend for increased plasma CRP levels in the depressed FMT rats (p = 0.083), but no significant differences in **(h)** IL1b (p = 0.09), **(i)** IL-6 (p = 0.86), **(j)** TNF-α, (p = 0.57). **(k)** There were no significant differences in Bdnf gene expression between the groups (p = 0.75) and no significant differences in **(l)** plasma LBP Lipopolysaccharide binding protein (LBP) levels or **(m)** plasma corticosterone levels. **(n)** Rats that received the depressed FMT demonstrated a significant increase in intestinal transit time (p = 0.013).

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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