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RESEARCH ARTICLE

Dysosmobacter welbionis J115^T reduces stress-like phenotype in high-fat diet-induced obese female mice

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Abstract

This study investigated the effects of *Dysosmobacter welbionis* J115^T on stress- and anxiety-related behaviours, inflammation, and neurobiological markers under different dietary conditions in female mice. Daily oral gavage with *D. welbionis* J115^T for six weeks did not significantly impact body weight or fat mass, regardless of dietary treatment. Notably, high-fat diet (HFD)-fed female mice displayed increased body weight and adipose tissue accumulation compared to control diet (CTD) counterparts; however, this was not significantly altered by *D. welbionis* J115^T administration. Behavioural testing revealed that HFD-fed female mice exhibited a mild stress/anxiety-like phenotype, especially in the elevated plus maze (EPM) and forced swim test (FST), which was attenuated by *D. welbionis* J115^T treatment. These mice showed increased exploratory behaviour in the light-dark test (LDT), reduced time spent in closed arms of the EPM, and longer cumulative time in a highly active state in the FST. Plasma corticosterone levels, elevated post-behavioural testing in all female groups, increased less in HFD-fed *D. welbionis*-treated mice, suggesting a blunted stress response. These findings highlight sex-specific behavioural and molecular responses to dietary and probiotic interventions and suggest that *D. welbionis* J115^T may modulate stress-related behaviours in female mice via the gut-brain axis.

Keywords

Dysosmobacter welbionis – stress-like phenotype – high-fat diet-induced obesity – female mice – probiotics

1 Introduction

Obesity has become a large rising global health concern (Okunogbe *et al.*, 2022), with nutritional intake playing a pivotal role. Diet is crucial in our neurodevelopmental stages as well as for long-term physical and mental health (Adan *et al.*, 2019; Smyth *et al.*, 2015; Yao *et al.*, 2021). The gastrointestinal tract provides an environment for dietary compounds and the gastrointestinal microbiota to interact and microbial metabolites can be used to relay information in the form of neurotransmitters towards the brain demonstrating one of the several pathways for communication along the gut-microbiota-brain axis (Cryan and Dinan, 2012; Hsiao *et al.*, 2013; Mittal *et al.*, 2017). Aggregation of evidence towards involvement of the gut-microbiota-brain axis in neuro-

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logical diseases and disorders, such as Autism Spectrum Disorder (Hosie *et al.*, 2022), Major Depressive Disorder (Winter *et al.*, 2018), Parkinson's disease (Hassan *et al.*, 2025; Park *et al.*, 2025) and Alzheimer's disease (Li *et al.*, 2025; Shabbir *et al.*, 2021) have been identified. Additionally, the widespread Western diets (high in fat and sugars) have been linked to an increased risk of developing mental health disorders like depression (Gangwisch *et al.*, 2015; Le Port *et al.*, 2012). Other than dietary interventions or lifestyle changes, potential therapeutics include prebiotics, probiotics and even postbiotics. Probiotics have been used to fortify the intestinal barrier and influence the native microbiota communities in efforts to support host health (Anderson *et al.*, 2010; Blackwood *et al.*, 2017; Krumbeck *et al.*, 2018; Patel *et al.*, 2012).

Dysosmobacter welbionis has been recently identified as a bacterial biomarker in the gut microbiota of major depressive disorder patients (MDD), with these patients presenting with increased levels of *D. welbionis* (Wang *et al.*, 2025). Stress has also been identified as one of the major contributing factors towards depression (Koizumi, 1996). Furthermore, obesity has been found to increase the incidence of depression and anxiety (Andre *et al.*, 2014; Milaneschi *et al.*, 2017; Xu *et al.*, 2011; Zemdegs *et al.*, 2016). In an irritable bowel disease cohort, *D. welbionis* prevalence (not abundance) was negatively correlated with ulcerative colitis and Crohn's disease (Nickols *et al.*, 2024). Nickols *et al.* (2024) concluded that the role of *D. welbionis* in gut health may be invoked purely due to presence (regardless of abundance) and that *D. welbionis* may be sensitive to intestinal inflammation leading to undetectable traces of *D. welbionis* in IBD. *D. welbionis* has also been demonstrated to counteract diet-induced obesity, reduce adipose tissue and intestinal inflammation in a high-fat diet mouse model of obesity (Le Roy *et al.*, 2022; Le Roy *et al.*, 2020; Moens de Hase *et al.*, 2024). *D. welbionis* has also been inversely associated with weight gain in HIV patients treated with integrase inhibitor-based antiretroviral therapy (De Greef *et al.*, 2025). Therefore, in this study we investigated the effects of *Dysosmobacter welbionis* J115 in a mouse model of diet-induced obesity, concentrating our efforts on investigating stress/anxiety-related behaviour specifically in female mice that tend to display a more pronounced depressive-like phenotype than their male counterparts (Pitzer *et al.*, 2022).

2 Materials & methods

Ethics

Mouse experiments were approved by the Ethical Committee for Animal Care of the Health Sector of the Université catholique de Louvain (UCLouvain), headed by Prof. J-P Dehoux, under number 2022/UCL/MD/41. The experiments were performed in accordance with the guidelines of the Local Ethics Committee and in compliance with the Belgian Law of 29 May 2013 regarding the protection of laboratory animals (agreement number LA1230314).

Animals

Seven-week-old female C57BL6/JRj SOPF mice (n = 48) were obtained from Janvier laboratories (Saint-Berthevin, France). Animals were group-housed (three animals per cage) under standard conditions (room temperature 22 ± 2 °C; 12-h light-dark cycle) with ad libitum access to irradiated food and autoclaved milliQ water. Cage enrichment included a gummy gnawing bone (Bio-Serv; Flemington, NJ, USA), a red mouse house (Tecniplast; Provincia di Varese, Italy) and paper towel-based bedding material. All animals were acclimatised in our SOPF facility for one week prior to starting experimentation.

The mice were split into either the control low-fat diet (CTD: AIN-93M mature rodent diet; 9.4 kcal% fat, 20 kcal% protein and 20 kcal% carbohydrate; D10012Mi, Research Diets, New Brunswick, NJ, USA) or the high-fat diet (HFD: rodent diet with 60 kcal% fat; 20 kcal% protein and 20 kcal% carbohydrate, D12492i, Research Diets, New Brunswick, NJ, USA). In both diets, the fat content is composed of soybean oil – with the addition of lard in the HFD to create a 60 kcal% fat diet; sucrose and maltodextrin (lodex 10) – with the addition of corn starch in the CTD – comprises the carbohydrate content in the diets, for complete diet details see ESI (Table 1). These mice were further allocated to one of the gavage treatments: (1) vehicle (trehalose) gavage and (2) live *Dysosmobacter welbionis* J115^{T} (1×10^{9} CFUs/200 ml gavage volume); resulting in the experimental female groups: CTD + vehicle, CTD + *D. welbionis* J115; HFD + vehicle, HFD + *D. welbionis* J115; and experimental male groups: CTD + vehicle, CTD + *D. welbionis* J115 (n = 12 for each group).

The mice were weighed daily and body composition analysis was performed using a 7.5 MHz time domain-nuclear magnetic resonance machine (LF50 Minispec; Bruker, Rheinstetten, Germany) at three time points during the experiment: (1) baseline – before diet/gav-

TABLE 1 Experimental dietary details of the control diet (D10012Mi) and high fat diet (D12492i, 60 kcal% fat)

Class description	Ingredients	Weight (g)
D10012Mi		
AIN-93M mature rodent diet		
Protein	Casein, Lactic, 30 Mesh	140.00
Protein	Cystine, l	1.80
Carbohydrate	Starch, Corn	495.69
Carbohydrate	Lodex 10	125.00
Carbohydrate	Sucrose, Fine Granulated	100.00
Fiber	Solka Floc, FCC200	50.00
Fat	Soybean Oil, USP	40.00
Mineral	S10022M	35.00
Vitamin	V10037	10.00
Vitamin	Choline Bitartrate	2.50
Anti-oxidant	tert-Butylhydroquinone (tBHQ)	0.01
D12492i		
Rodent diet with 60 kcal% fat		
Protein	Casein, Lactic, 30 Mesh	200.00
Protein	Cystine, l	3.00
Carbohydrate	Lodex 10	125.00
Carbohydrate	Sucrose, Fine Granulated	72.80
Fiber	Solka Floc, FCC200	50.00
Fat	Lard	245.00
Fat	Soybean Oil, USP	25.00
Mineral	S10026B	50.00
Vitamin	Choline Bitartrate	2.00
Vitamin	V10001C	1.00
Dye	Dye, Blue FD&C #1, Alum. Lake 35-42%	0.05

Macronutrient	kcal%
D10012Mi	
AIN-93M mature rodent diet	
Protein	20
Carbohydrate	20
Fat	9.4
D12492i	
Rodent diet with 60 kcal% fat	
Protein	20
Carbohydrate	20
Fat	60

age intervention, (2) pre-behavioural testing and (3) post-behavioural testing – on the day of sacrifice. At the end of each experiment, mice were anaesthetised with isoflurane (Forene, England). After the mice were euthanized; various adipose tissues were isolated and weighed, and brain tissue (hippocampus, hypothalamus and cortex) was promptly collected, snap-frozen with liquid nitrogen and stored at –80 °C until further processing.

Plasma corticosterone ELISA

At three time points, i.e. (1) before experimental diet/gavage, (2) pre-behavioural battery testing and (3) post-behavioural battery testing, ~75 µl of tail vein blood was collected using haematocrit capillary tubes, promptly processed and stored at –80 °C. Plasma corticosterone measurements were determined using a corticosterone ELISA kit (Enzo life sciences, USA) with a detection range from 32 to 20,000 pg/ml; and was carried out according to manufacturer's instructions but with an initial 1:30 dilution according to the small volume protocol for serum/plasma. Samples were run in duplicate and the absorbance measured at 405 nm (with 570 nm correction) using a SpectraMax i3× microplate reader (Molecular Devices, USA).

RNA extraction and analyses

RNA was extracted from brain tissue using Trizol Isolation Reagent (Roche; Basil, Switzerland) with a single 5 mm stainless steel bead and the TissueLyser II machine (Qiagen) at 30 Hz for 2 min. Extracted RNA was converted into cDNA using the GoScript Reverse Transcriptase kit (Promega; WI, USA). The QuantStudio 3 RT PCR system (Thermo Fisher; Waltham, MA, USA) was used to carry out Real-Time PCR with SYBR Green (Promega) with the housekeeper gene RPL19 and genes of interest. Primers and sequences are provided in ESI (Table 2). Samples were processed in duplicate with the following thermocycling conditions: 95 °C for 2 min, 40 cycles of: 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s; 95 °C for 1 s, 60 °C for 20 s and finally 95 °C for 1 s. Results were analysed using the 2-ΔΔCT method.

Behavioural tests

All materials were cleaned with 70% ethanol before testing. All mice were habituated in their home cage for at least one hour inside the behaviour room to acclimatise before commencing behavioural testing. Behavioural tests were carried out in order of impact (lowest to the highest): open field test, marble bury test, light-dark test, elevated plus maze, and forced swim test. To

TABLE 2 qPCR primer sequences used to investigate inflammation and barrier function in the mouse brain (cortex, hippocampus and hypothalamus)

Gene name	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
BDNF	GCG-CCC-ATG-AAA-GAA-GTA-AA	GTC-GTC-AGA-CCT-CTC-GAA-CC
Claudin 2	AAG-GTG-CTG-CTG-AGG-GTA-GA	AGT-GGC-AGA-GAT-GGG-ATT-TG
Claudin1	TTC-GCA-AAG-CAC-CGG-GCA-GAT-ACA	GCC-ACT-AAT-GTC-GC-AGA-CC-GAA-A
IL-1b	TCG-CTC-AAG-GTC-ACA-AGA-AA	CAT-CAG-AGG-CAA-GGA-GGA-AAA-C
IL-6	ACA-AGT-CGG-AGG-CTT-AAT-TAC-ACA-T	TTG-CCA-TTG-CAC-AAC-TCT-TTT-C
MCP1	GGC-TGG-AGA-GCT-ACA-AGA-GG	GCT-GAA-GAC-CTT-AGG-GCA-GA
Occludin	ATG-TCC-GGC-CGA-TGC-TCT-C	TTT-GGC-TGC-TCT-TGG-GTC-TGT-AT
RPL19	GAA-GGT-CAA-AGG-GAA-TGT-GTT-CA	CCT-TGT-CTG-CCT-TCA-GCT-TGT
TLR4	CCC-TCA-GCA-CTC-TTG-ATT-GC	TGC-TTC-TGT-TCC-TTG-ACC-CA
TNFa	AGC-CCC-CAG-TCT-GTA-TCC-TT	GGT-CAC-TGT-CCC-AGC-ATC-TT
ZO1	TTT-TTG-ACA-GGG-GGA-GTG-G	TGC-TGC-AGA-GGT-CAA-AGT-TCA-AG

reduce stress from the behavioural test battery, a 48-h rest period was established between each behavioural test. A GigE monochrome Basler camera and Ethovision XT software (v17.0.1630, Noldus Information Technology; Wageningen, Netherlands) were used to capture and assess mouse behaviour. The experimenter was blinded to the experimental group during the tests and was only unblinded after completion of the analysis. The behavioural tests (open field test, marble bury test, light dark test, elevated plus maze and forced swim test) were carried out as described previously (Wong *et al.*, 2025).

Dysosmobacter welbionis *J115^T culturing*

D. welbionis J115^T was cultured anaerobically in a modified YCFA medium supplemented with myo-inositol (10 g/l). At harvesting, the culture media was centrifuged at 5,000 × *g* for 15 min, with the resulting cell pellet resuspended in a 15% trehalose solution and stored in anaerobic vials at –80 °C. Colony forming unit counts were carried out to determine the number of live bacteria in the vials used for oral gavage administration. The 15% trehalose solution for cryoprotection was also used as the vehicle gavage solution.

Statistical analysis

All behavioural tests were captured with a GigE monochrome Basler camera lens and analysed using Noldus EthoVision XT (v17; (Noldus *et al.*, 2001)). Statistical tests of the behavioural test results were analysed with R (version 4.1.1; RCoreTeam, 2019) and GraphPad Prism (version 10; GraphPad Software). If the data satisfied the assumptions for an ANOVA, a two-way ANOVA and Tukey multiple comparison correction were carried out.

3 Results

Daily oral administration of D. welbionis *J115^T did not affect weight or adiposity in female mice after six weeks, regardless of diet*

As expected, significantly greater body weight and fat mass was observed in HFD-fed female mice compared to the CTD-fed female mice; but no significant differences with daily administration of *Dysosmobacter welbionis* J115^T was observed within both the HFD and CTD-fed female mouse cohort, respectively (Figure 1A & B). Female mice on HFD with vehicle gavage treatment had a significantly increased total body weight compared to their counterparts on the CTD starting at week five (week five P-value = 0.0182; week six P-value ≤ 0.0001), whereas this divergence between the two diets in the mice administered *D. welbionis* J115^T only began one week later (week six; P-value = 0.0016; Figure 1A). At the end point of the experiment there was a difference of 1.12 g of average total adipose tissue weight within the HFD-fed female mice groups (P-value = 0.47; Figure 1C). This difference mainly persisted due to the subcutaneous adipose tissue in female HFD-fed mice with or without *D. welbionis* J115^T gavage (0.38 g difference, P-value = 0.016). At sacrifice, comparing the experimental diets within the same gavage treatment yielded differences only in the subcutaneous (P-values ≤ 0.0001, <0.0001) and ovarian adipose tissue (vehicle gavage P-value ≤ 0.0001, *D. welbionis* J115^T P-value = 0.0033) in female mice; with no differences in visceral adipose tissue weight between any groups (Figure 1C).

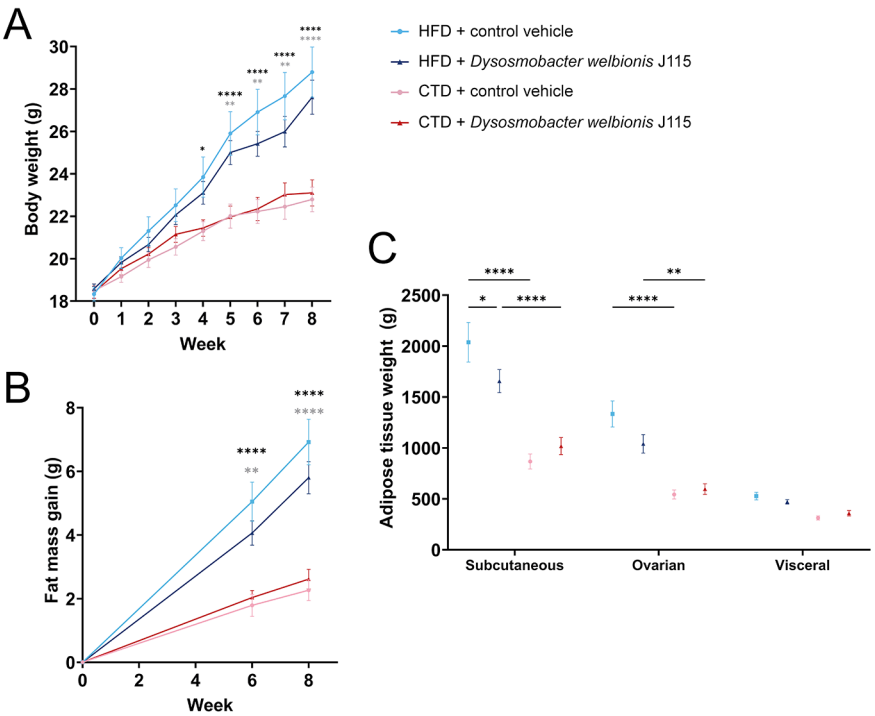

FIGURE 1 Seven-week-old female C57BL/6JRj mice were placed on either a control (CTD) or high-fat diet (HFD) and were treated with either a control vehicle or *Dysosmobacter welbionis* J115 (1×10^{10} CFUs) by daily oral gavage. Body weight (A), fat mass (B) and adipose tissue: subcutaneous, gonadal (epididymal/ovarian) and visceral weights (C). All data are shown as mean ± SEM. For each experimental group, n = 12. Data were assessed using a two-way ANOVA with Tukey multiple comparison correction. Black asterisks denote significant *P*-value comparisons between female mice with the control vehicle gavage (CTD vs HFD) and grey asterisks between the *Dysosmobacter welbionis* J115 gavage groups (CTD vs HFD). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Plasma corticosterone is elevated post-behavioural testing, with attenuation in D. welbionis *J115-treated HFD females*

Corticosterone levels were evaluated at three time points: baseline – before starting gavage and dietary treatment (1), before (2) and after (3) the behavioural testing period. Overall, when comparing between the experimental groups within each time point, there are no significant differences observed; yet when considering one experimental group across the three time points, we observed all four female mice groups had a significantly increased plasma corticosterone level at the end of behavioural testing compared to baseline (prior to starting the experimental diet/gavage; CTD + vehicle $P = 0.0079$; CTD J115 $P = 0.0002$; HFD + vehicle $P = 0.0014$; HFD J115 $P = 0.0137$; Figure 2).

BDNF expression in the cortex was significantly more expressed (*P*-value = 0.039) in mice on HFD with *D. welbionis* J115^T gavage compared to mice on HFD with vehicle gavage (Figure 3A). Similarly, the CTD + *D. welbionis* J115^T group had increased expression of IL-1ß in the hippocampus compared to the CTD + vehicle group ($P = 0.00176$; Figure 3B). The brain barrier genes tested (CL1, CL2, OCC, ZO1) did not have any differences between the female experimental groups for the three brain regions.

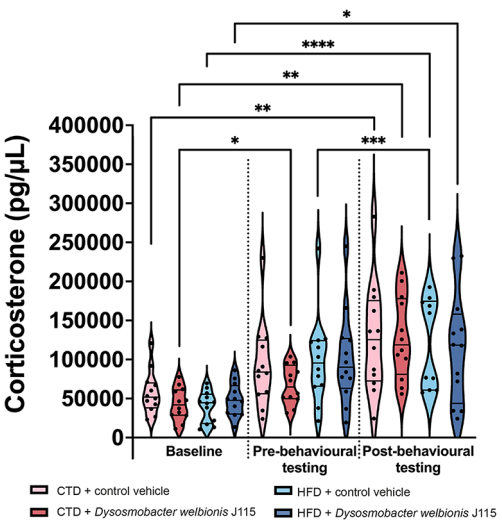

FIGURE 2 Plasma corticosterone levels measured at three time points: (1) baseline (pre-gavage/diet intervention; (2) six weeks post-gavage/diet intervention pre-behavioural testing and (3) after behavioural battery testing. Data are shown as violin plot. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

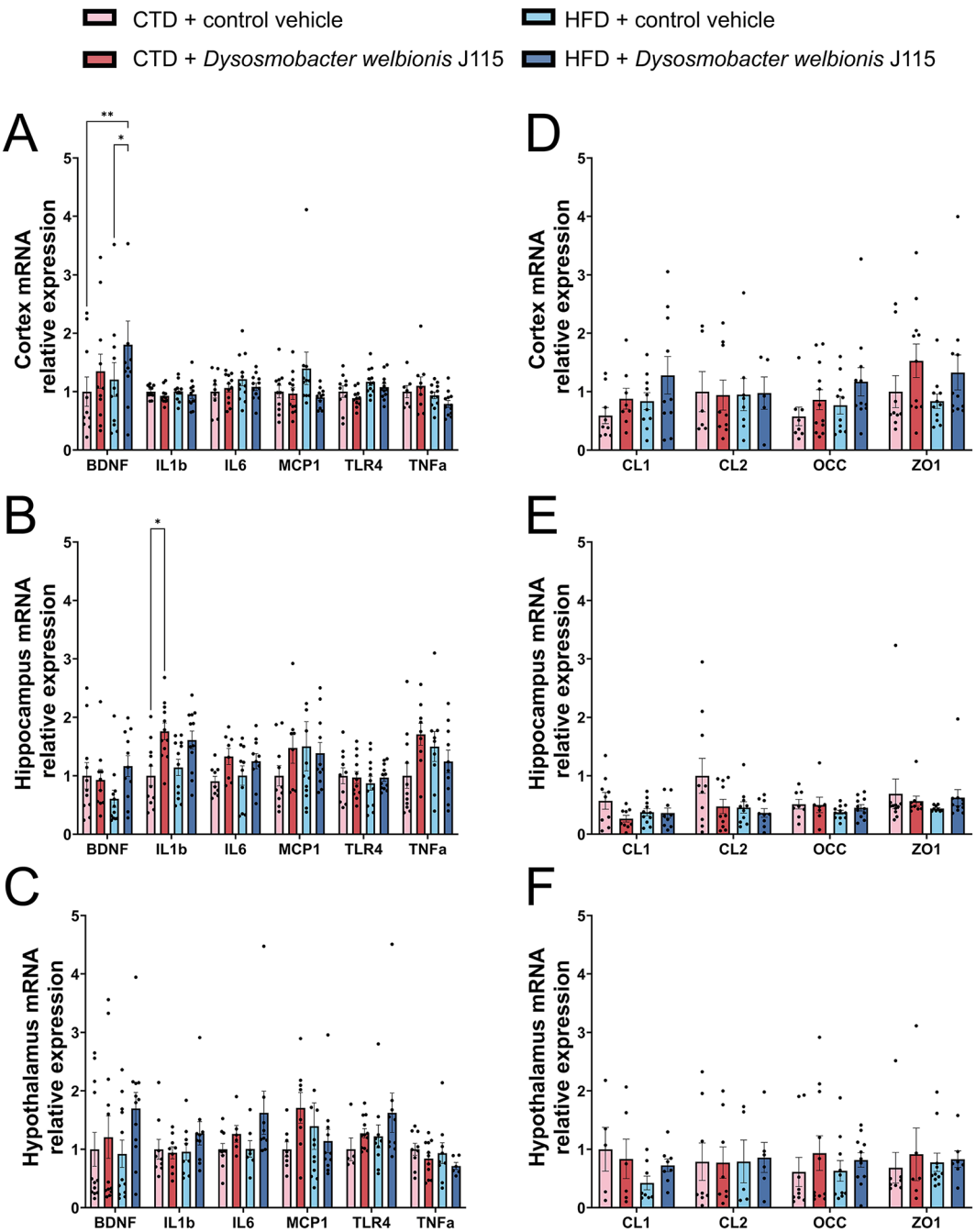

FIGURE 3 Brain cortex, hippocampus and hypothalamus qPCR results for barrier (CL-1, CL-2, OCC, ZO-1) and inflammation (BDNF, IL-1ß, IL-6, MCP1, TLR4, TNFα) genes. Experimental groups were assessed for outliers with ROUT and a two-way ANOVA with Tukey multiple comparison correction was carried out for group comparisons.

HFD-fed female mice exhibit a mild stress/anxiety-like phenotype that is attenuated by D. welbionis *J115^T administration*

Although there were no differences found in the male mice on CTD with or without *D. welbionis* J115^T for the behavioural testing battery (data not shown), we identified several differences between female CTD and HFD with vehicle gavage mice in the EPM cumulative duration not moving (P = 0.0080, CTD mean = 3.663 m, HFD mean = 1.783 m) (Figure 4). Most significant differences between these two groups were observed in the forced swim test, with a significantly increased time spent mobile in HFD mice (P = 0.0013; CTD = 20.58 s, HFD =10.88 s), cumulative duration immobile (P = 0.0013; CTD = 339.4 s, HFD = 349.1 s) and frequency in the mobility state (P = 0.0044; CTD = 316.9; HFD = 219.3) (Figure 4).

We determined significant differences in behaviour between the female mice treated with *D. welbionis* J115^T. In the light dark test (Figure 4A-C): the HFD J115

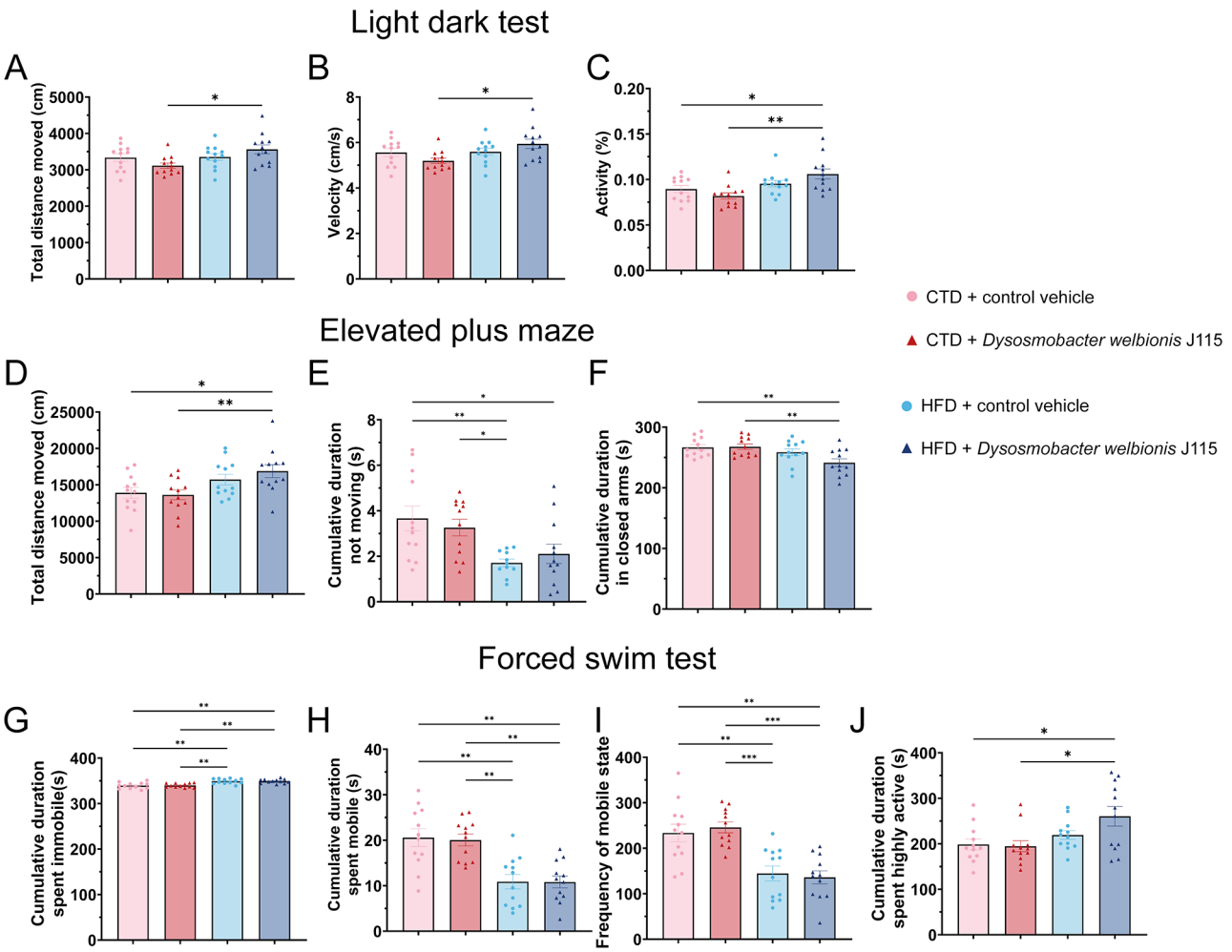

FIGURE 4 A selection of mouse behavioural testing outcomes from the behavioural battery testing of open field, marble bury, light-dark, elevated plus maze and forced swim. Each experimental group n = 12, with outlier detection and removal carried out by ROUT and a two-way ANOVA with Tukey multiple comparison correction for group comparisons. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

group displayed the greatest mean total distance moved (3,562 cm) compared the CTD J115 group (3,115 cm; $P = 0.0208$), velocity ($P = 0.0205$; CTD J115 mean = 5.193 cm/s, HFD J115 mean = 5.939 cm/s) and percentage of time spent active ($P = 0.0015$, CTD J115 = 8.1%; HFD J115 = 10.6%). In the elevated plus maze (Figure 4D-F): total distance moved ($P = 0.0068$, CTD J115 = 13,617 cm; HFD J115 = 16,880 cm), velocity ($P = 0.0074$; CTD J115 = 37.84 cm/s; HFD J115 = 47.22 cm/s) and cumulative duration in closed arms ($P = 0.0031$; CTD J115 = 267.7 s; HFD J115 = 241.4 s) were observed. Additionally, the assessment of three different mobility states during the forced swim test resulted in the HFD-fed + *D. welbionis* J115^T spending the greatest cumulative time in a highly active state compared to its CTD-fed counterpart and even beyond the baseline group on the CTD-fed + vehicle gavage ($P = 0.0183$; CTD = 198.6 s, CTD J115 = 194.7 s, HFD J115 = 260.5 s) also seen in the ratio between active: inactive activity ($P = 0.035$) and in the frequency of a mobile state ($P \leq 0.0001$; CTD J115 = 338.8; HFD J115 = 198.8) (Figure 4G-J). Therefore, administration of *D. welbionis* J115^T in HFD-fed mice increased exploratory behaviour in the LDT, decreased time spent in the closed arms of the EPM; and they spent more time in a highly active state with fewer changes between the immobile and mobile state in the FST; overall indicating a reduction in anxiety-like behaviour with *D. welbionis* J115^T treatment in female mice between the two diets.

4 Discussion

In the literature, obesogenic diets and chronic intestinal inflammation has been associated with anxiety-related behaviours (Bercik *et al.*, 2010; Maltz *et al.*, 2022). Obese mice have also demonstrated varying results in the open

field test, ranging from reduced locomotion, no difference or increased locomotion (Eudave *et al.*, 2018; Mort *et al.*, 2023; Sharma and Fulton, 2013). In particular, female mice fed an obesogenic diet exhibit an increase of anxiety-related behaviour in the EPM and OFT between 11 and 13 weeks of age (Mort *et al.*, 2023) and female mice tend to exhibit stronger phenotypical differences in the FST (Kokras *et al.*, 2015). We found differences between CTD and HFD-fed female mice with vehicle gavage in the EPM and FST, but notably in the J115 treated mice, we observed a significant reduction of time spent in the closed arms of the EPM and also an increased cumulative time spent highly active with a reduction of changing between the active and inactive state in the FST.

Our data suggest that the behavioural effects of *D. welbionis* J115^{T} may be mediated via the gut-brain axis. A possible mechanism involves microbial production of short-chain fatty acids (SCFAs) such as butyrate, which are known to exert anti-inflammatory effects and influence host metabolism, including glucose and lipid homeostasis (Chen and Vitetta, 2020; Morrison and Preston, 2016; Portincasa *et al.*, 2022). Previous work has shown that *D. welbionis* J115^{T} utilises myo-inositol to produce butyrate and can reduce adiposity and improve glucose control in HFD-fed mice (Le Roy *et al.*, 2020). Although we did not measure SCFAs in this study, the observed increase in BDNF expression and reduction in stress-related behaviours align with previously described effects of butyrate in models of depression and anxiety.

Sodium butyrate has been used as an antidepressant drug in mouse studies and results in the decrease of immobility in the tail suspension test and also a 50% increase of prefrontal cortex and hippocampus levels of BDNF (Schroeder *et al.*, 2007; Sun *et al.*, 2016; Valvassori *et al.*, 2014; Valvassori *et al.*, 2024); associating BDNF transcript levels in the prefrontal cortex with an antidepressant-like behavioural response. Decreased levels of BDNF was discovered in the frontal cortex and hippocampus of suicide patients post-mortem (Dwivedi *et al.*, 2003) and significantly increased levels of BDNF in these brain regions were also observed in patients with antidepressant administration (Einat *et al.*, 2003). Administering the potential probiotic *D. welbionis* J115^{T}, resulted with increases of BDNF expression in the prefrontal cortex, hippocampus and hypothalamus for both CTD and HFD female mice but this increase was only significantly different in the prefrontal cortex of HFD-fed mice. This increase in cortical BDNF expression in the HFD-fed J115-treated mice coincided with the most marked improvements in behavioural outcomes observed in the elevated plus maze and forced swim test. While causality cannot be inferred, this alignment raises the possibility that the anxiolytic- and antidepressant-like effects of *D. welbionis* J115^{T} may, at least in part, be mediated through the upregulation of BDNF in the brain. Upregulation of OCC and ZO-1 hippocampal levels have also been shown as an effect of sodium butyrate treatment in chronic unpredictable mild stress mouse models (Cristiano *et al.*, 2022; Sun *et al.*, 2016), potentially partially restoring blood-brain barrier integrity, but this effect was not observed in our study.

As we only extracted RNA from the brain samples and did not preserve tissue for protein analysis, we were unable to assess BDNF and other markers in the different brain regions at the protein level. Confirming these results through protein quantification would be an important next step.

Stress response has also been shown to be affected by commensal gut microbes which are able to be modulated in germ free mice with SPF mouse faecal transplantation (Sudo *et al.*, 2004). Modulation of the gut microbiota via probiotics have also shown to have beneficial effects towards reducing brain inflammation (D'Mello *et al.*, 2015) and ameliorating anxiety and depression-like behaviour in mice (e.g. with *Bifidobacterium longum*) (Tamayo *et al.*, 2025). While *D. welbionis* has been identified as a gut microbiome biomarker of major depressive disorder (Wang *et al.*, 2025), investigation of individual gut microbiome markers warrants greater investigation into the causal affiliation with markers identified by correlation associations. Our data do not support a causal role for increased *D. welbionis* in promoting depressive-like behaviour, contradicting what might be inferred from biomarker-based studies. These data are rather aligned with previously reported antidepressant effects of butyrate producing bacteria.

The sex-dependent differences identified here and in our previous study (Wong *et al.*, 2025), particularly in behaviour, demonstrates the importance of using both genders in the detection of gender differential phenotypes but also their potential responses to probiotic treatment. While we did not observe significant differences in all the behavioural tests, there is some evidence towards a reduction of stress/anxiety-related behaviour in female mice.

Perhaps by utilising a chronic stress model or introducing multiple stress related events (Antoniuk *et al.*, 2019; Picard *et al.*, 2021) in addition to extending the time period of high fat diet-feeding and including a high

fat high sucrose fed group in parallel, would to aid to produce a stronger stress/anxiety related phenotype to more precisely allow us to test our probiotic – especially if this generates the presence of a strong phenotype to rescue.

5 Conclusions

Overall, we demonstrated that the diet-induced obesity model (60% kcal fat diet) in male mice failed to produce a strong stress/anxiety related phenotype (Wong *et al*., 2025); whereas in this study using female mice we observed a greater inclination towards this phenotype most distinctly in the elevated plus maze and forced swim test.

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Authors' contribution

GCW, MVH, PDC conceptualised the experiments; PDC acquired funding for this project; NMD and PDC provided the MNUT animal and molecular biology platforms. GCW performed all experimentation and statistical analyses. GCW, MVH, PDC wrote, reviewed and edited the manuscript. All authors approved the manuscript.

Conflict of interest

PDC is inventor on patent applications dealing with the use of gut bacteria and their components in the treatment of diseases. PDC was a co-founder of Enterosys.

Data availability

The original contributions presented in the study are included in the article/ESI, further inquiries can be directed to the corresponding author.

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