CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	Volatility as a concept to understand the impact of stress on the microbiome
Authors	Bastiaanssen, Thomaz F. S.;Gururajan, Anand;van de Wouw, Marcel;Moloney, Gerard M.;Ritz, Nathaniel L.;Long-Smith, Caitriona M.;Wiley, Niamh;Murphy, Amy;Lyte, Joshua M.;Fouhy, Fiona;Stanton, Catherine;Claesson, Marcus J.;Dinan, Timothy G.;Cryan, John F.
Publication date	2021-02-01
Original Citation	Bastiaanssen, T. F. S., Gururajan, A., van de Wouw, M., Moloney, G. M., Ritz, N. L., Long-Smith, C. M., Wiley, N., Murphy, A., Lyte, J. M., Fouhy, F., Stanton, C., Claesson, M. J., Dinan, T. G. and Cryan, J. F. (2021) 'Volatility as a concept to understand the impact of stress on the microbiome', Psychoneuroendocrinology, 124, 105047 (13pp). doi: 10.1016/j.psyneuen.2020.105047
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.psyneuen.2020.105047
Rights	© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) - https://creativecommons.org/licenses/by/4.0/
Download date	2026-11-04 07:39:36
Item downloaded from	https://hdl.handle.net/10468/13033

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Psychoneuroendocrinology 124 (2021) 105047

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Volatility as a Concept to Understand the Impact of Stress on the Microbiome

Thomaz F.S Bastiaanssen [a,b,1], Anand Gururajan [a,b,1], Marcel van de Wouw [a,b], Gerard M Moloney [a], Nathaniel L Ritz [a,b], Caitriona M Long-Smith [a,b], Niamh C Wiley [c], Amy B Murphy [c,d], Joshua M Lyte [a], Fiona Fouhy [b,c], Catherine Stanton [b,c], Marcus J Claesson [b,d], Timothy G Dinan [b,e], John F Cryan [a,b,*]

[a] Department of Anatomy & Neuroscience, University College Cork, Ireland
[b] APC Microbiome Ireland, University College Cork, Ireland
[c] Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland
[d] School of Microbiology, University College Cork, Ireland
[e] Department of Psychiatry & Neurobehavioural Science, University College Cork, Ireland

ARTICLE INFO

Keywords:
Chronic stress
Microbiome
Gut-brain axis
Volatility
Corticosterone

ABSTRACT

The microbiome-gut-brain-axis is a complex phenomenon spanning several dynamic systems in the body which can be parsed at a molecular, cellular, physiological and ecological level. A growing body of evidence indicates that this axis is particularly sensitive to the effects of stress and that it may be relevant to stress resilience and susceptibility. Although stress-induced changes in the composition of the microbiome have been reported, the degree of compositional change over time, which we define as volatility, has not been the subject of in-depth scrutiny. Using a chronic psychosocial stress paradigm in male mice, we report that the volatility of the microbiome significantly correlated with several readouts of the stress response, including behaviour and corticosterone response. We then validated these findings in a second independent group of stressed mice. Additionally, we assessed the relationship between volatility and stress parameters in a cohort of health volunteers who were undergoing academic exams and report similar observations. Finally, we found inter-species similarities in the microbiome stress response on a functional level. Our research highlights the effects of stress on the dynamic microbiome and underscores the informative value of volatility as a parameter that should be considered in all future analyses of the microbiome.

1. Introduction

The mammalian gut plays host to approximately 1 trillion microbial organisms collectively known as the gut microbiome (Sommer and Bäckhed, 2013). The microbiome is highly sensitive and reactive to the effects of stress to the extent that it is now accepted that the stress response is not solely the domain of brain function, but rather that it results from a synergy of mechanisms that constitute the gut-brain axis (Bastiaanssen et al., 2018; Bastiaanssen et al., 2020; Cruz-Pereira et al., 2020; Cryan et al., 2019; Dinan and Cryan, 2012; Foster et al., 2017). In particular, studies in rodents have correlatively linked alterations in microbiota composition to the effects of stress on behaviour (Bharwani et al., 2016; Bharwani et al., 2017; Burokas et al., 2017; Marin et al., 2017; Szyszkowicz et al., 2017; Xu et al., 2020) and the central/-peripheral inflammatory milieu (Bailey et al., 2011; Bharwani et al., 2016; Bharwani et al., 2017; Burokas et al., 2017; Szyszkowicz et al., 2017). Going further, manipulation and perturbation of the microbiome have been shown to alter the reaction to stress, further solidifying the regulatory role of the microbiome in the stress response (Donoso et al., 2020; Jašarević et al., 2017; Kuti et al., 2020; Langgartner et al., 2018; Morais et al., 2020; Pearson-Leary et al., 2019; Provensi et al., 2019; Stothart et al., 2019; Wang et al., 2020). Moreover, in humans there have been a number of studies confirming a relationship between stress and microbiome composition across the lifespan (Allen et al., 2016; Hemmings et al., 2017; Messaoudi et al., 2011; Papalini et al., 2019; Zijlmans et al., 2015).

* Corresponding author at: Department of Anatomy & Neuroscience, University College Cork, Western Road, Cork, Ireland.
E-mail address: j.cryan@ucc.ie (J.F. Cryan).
[1] Co-first author.

https://doi.org/10.1016/j.psyneuen.2020.105047
Received 25 March 2019; Received in revised form 29 October 2020; Accepted 3 November 2020
Available online 8 December 2020
0306-4530/© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

These observations notwithstanding, a common feature of the above cited studies is the fact that they are a 'snapshot-in-time' analyses of samples and therefore do not consider the compositional and temporal instability of the microbiome (Caporaso et al., 2011; Claesson et al., 2011). Temporal variance as a feature of the microbiome has been discussed before. Initially, the volatile nature of the microbiome was pointed out (Weinstock, 2011) and relatively soon thereafter, the term volatility was used to refer to the degree of change between timepoints (Goodrich et al., 2014). Aside from these studies, the term volatility has been scarcely used in the context of the microbiome. One example of its rare use is in the context of the rapidly shifting microbial communities on shoes and cell phones (Lax et al., 2015). In the context of stress, recent studies have shown differential effects on microbiota beta-diversity dependent on when the samples were taken – shortly after commencement of social stress or towards the end of the protocol (Gautam et al., 2018), though the degree of change was not pursued, nor linked to the severity of the stress response. More broadly, there have been reports of microbiome beta-diversity being altered after stress compared to control, though often these reports do not relate severity of stress to difference in beta diversity, nor do they take the baseline microbiome into account (Langgartner et al., 2018; Xu et al., 2020). Recently, in a large human cohort, patients with inflammatory bowel disease (IBD), a condition that is associated with an increased level of stress, were shown to have a more volatile microbiome than healthy volunteers (Clooney et al., 2020; Ryan et al., 2020). While these studies provide some evidence that volatility may be related to stress, the impact of stress on microbial volatility measures remains unknown. Furthermore, deciphering what makes a microbial community volatile can provide new insights into its role in mediating the effects of stress. To further investigate volatility in relation to stress, we exposed mice to chronic psychosocial stress and assessed volatility. Based on the findings of this discovery cohort, we then set out to validate our findings in an independent cohort of a different strain of mice under the same experimental conditions. Finally, we also took advantage of an ongoing study involving healthy volunteers undergoing academic examination stress and sought to examine whether the interaction between volatility and stress occurred across species.

2. Materials & Methods

2.1. Animals

For this study we used two cohorts of adult male mice. The first cohort will be referred to as the Discovery cohort and consisted of (B6;129-*Gt(ROSA)26Sor*$^{tm1(CAG-cas9,-EGFP)Fezh}$/J; https://www.jax.org/strain/024857). The second cohort will be referred to as the Validation cohort and consisted of adult male C57BL/6 (Envigo, UK). There were no differences in terms of experimental treatment and handling between the two cohorts. There were three weeks between the arrival of the animals and the start of singly housing. Approximately one week before commencement of social defeat sessions, all mice were singly housed and weighed daily over the course of the experimental protocol (Fig. 1). For the chronic social defeat stress procedure, non-experimental singly housed adult male CD1 were used as aggressors (Envigo, UK). Mice were kept under a 12 hr light/dark cycle (ON 7:30AM, OFF 7:30PM) in a temperature/humidity controlled environment (21 °C, 55.5%) with food and water *ad libitum*. The main behavioural and physiological responses to chronic stress of the Discovery cohort have been initially reported elsewhere (Gururajan et al., 2019) and are used here in a correlative capacity with the microbiome analysis.

2.2. Chronic social defeat stress

Mice were randomly assigned to either the social defeat stress or control groups. Defeat sessions were performed as previously described (Gururajan et al., 2019). Briefly, for each defeat session, test mice were subjected to a pre-screened aggressor CD1 mouse until the first attack, expression of submissive posturing or until 5 min had passed. The mice were then separated by a perforated plexiglass wall that allowed only non-physical contact for 2 hrs. Subsequently, the separator was removed and, after another defeat, mice were transferred back to their home-cage. This was repeated with a different CD1 aggressor each day for 10 days. Control mice remained in their home-cages over the course of the stress protocol but were handled to an equal extent as the stressed mice in the process of measuring daily body weight and collecting tail-blood samples. Across the duration of the defeat protocol, to prevent contamination during defeat procedures, the experimenter removed any traces of faecal boli produced by the aggressor or the stressed mice. Based on the findings in the first cohort of mice, we repeated the experiment in a larger cohort. We refer to these cohorts as the Discovery cohort and the Validation cohort, respectively. Further details can be found in Supplementary Methods.

2.3. Social interaction test

The social interaction testing of mice was used to assess avoidance of the CD1 aggressors the day after the last defeat session and was carried out as previously described (Gururajan et al., 2019). Briefly, the test was carried out in a plastic box (41 × 32 × 24 cm) containing a wire mesh cage (9.5 × 7.5 × 7.0 cm) against one wall. In the first 2.5 min trial, the test mouse was allowed to explore freely. The mouse was then returned to its home cage for 1 min. During this time, an unfamiliar aggressor CD1 mouse was placed inside the wire mesh cage. This was followed by a second 2.5 min trial in which the test mouse was allowed to explore the area freely in the presence of the caged CD1. Social interaction (SIT) ratios were generated based on social investigation time in an arbitrarily defined interaction zone around the wire mesh cage. Further details can be found in Supplementary Methods.

2.4. Plasma sampling for corticosterone

Collection and analysis of plasma samples for corticosterone was carried out as previously described (Gururajan et al., 2019; van de Wouw et al., 2018). Briefly, tail bleeds were carried out within 1 hour of the lights turning off (1930-2030). Whole blood was collected in sterile eppendorfs. Whole blood was centrifuged (3500 g, 10 minutes, 4 degrees C) and plasma was collected. Plasma samples were analyzed in duplicate using the Enzo® Corticosterone ELISA kit plate (ADI-900-097, Enzo, Exeter, United Kingdom) according to the manufacturer's instructions. ELISA plates were read using a Multiskan® microplate photometer (Thermofisher Scientific®, Waltham, MA, USA) at 405 nm. See Supplementary Methods for further detail.

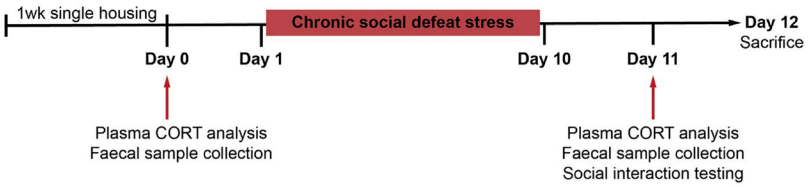


Fig. 1. Mouse experimental timeline. Mice were singly housed for 1 week prior to the first stool and plasma collection on Day 0. From Day 1 to 10, mice were randomly assigned to either the control condition (not shown) or chronic social defeat stress. On Day 11, plasma and faecal boli samples were again collected and social behaviour was assessed. The following day, all mice were culled. Trunk blood was collected for flow cytometry and brain tissue was processed for gene expression analyses.

2.5. Gene Expression Analysis

Gene expression analysis was carried out as previously described (Gururajan et al., 2019) Briefly, total RNA was extracted using the miRVana™ miRNA Isolation kit (Ambion/Life Technologies, Paisley, UK) according to the manufacturer's instructions. RNA was reverse-transcribed to complementary DNA using the Applied Biosystem® High Capacity cDNA Reverse Transcription Kit (10X RT Buffer, 25X dNTP mix (100 mM), 10X RT Random Primers, Multiscribe® Reverse Transcriptase) on the Applied Biosystem® GeneAmp PCR System 9700 (Thermofisher®, Waltham, MA, USA). qRT-PCR was carried out on the StepOnePlus® PCR machine (Thermofisher®, Waltham, MA, USA) using primer assays designed by Integrated DNA Technologies (Skokie, Illinois, USA). See supplementary information for primer IDs. Experimental samples were run in duplicates and to check for amplicon contamination, each run also contained template free controls for each probe used. PCR data were normalised using β-actin, with an average ct value of 20.9 (+/-0.5SD) across all samples, and transformed using the ΔΔCt method as previously described (Stilling et al., 2018).

2.6. Human Healthy Volunteer Study

Briefly, healthy volunteer study participants were recruited via advertisement and direct contact to the student population of University College Cork (UCC). A total of 84 volunteers responded to advertisement and direct contact; 54 were pre-screened by telephone call (64%); 36 were invited to a screening visit (43%); and thirty were enrolled in the study and randomised to treatment (36%). Inclusion criteria: participant must be able to give written informed consent; be between 18 and 30 years of age; be male; be in generally good health as determined by the investigator. Prior to testing days, participants were asked to refrain from strenuous exercise and alcohol 24 hours before the session, and from caffeine three hours prior to the session. At the screening visit, two weeks before baseline measurement, study participants were asked about their demographics, general medical history, medication record, and other metadata. Furthermore, the participants were screened using the MINI International Psychiatric Interview (to exclude subjects with a significant DSM-V psychiatric diagnosis). Participants attended for study visits during 2 semesters in UCC – both 8 weeks prior to an exam period and during an exam period. The exam visit took place during the participant's exams, but not on the day of an exam. The measures taken during the visit included Cohen's Perceived Stress Scale (PSS). Faecal samples from the morning of the visit were collected into plastic containers containing an Anaerogen sachet. Participants were instructed to keep the sample in a cool place until delivery at the study visit. Samples were immediately frozen at −80 °C at the visit. 200 mg was taken from the core after thawing and subsequently used for DNA extractions and 16 s sequencing. Salivary Cortisol Awakening Response was assessed using the Cortisol ELISA kit plate ADI-900-071, Enzo, Exeter, United Kingdom) according to the manufacturer's instructions. ELISA plates were read using a Multiskan® microplate photometer (Thermofisher Scientific®, Waltham, MA, USA) at 405 nm. These data were derived from control participants from a larger healthy volunteer study examining the effects of an intervention on the microbiota-gut-brain-axis (Moloney et al., 2020).

2.7. 16S rRNA Gene Sequencing

Faecal samples were collected during the weighing of mice before the first day and after the last day of social defeat. Boli were transferred to eppendorfs and stored at −80 °C. DNA was extracted from faecal samples and prepared for sequencing using an Illumina 16S Metagenomic Sequencing Library Protocol. See supplementary methods for further details.

2.8. Bioinformatics analysis

Three hundred base pair paired-end reads were pre-filtered based on a quality score threshold of >28 and trimmed, filtered for quality and chimaeras using the DADA2 library in R (version 3.6.3). Only samples with >10.000 reads after QC were used in analysis. Taxonomy was assigned with DADA2 against the SILVA SSURef database release v138. Parameters as recommended in the DADA2 manual were adhered to unless mentioned otherwise. ASVs were aggregated at genus level; those that were unknown on the genus level were not considered in downstream analysis, as were genera that were only detected as non-zero in 10% or few of total samples. As ratios are invariant to subsetting and this study employs compositional data analysis techniques (Aitchison et al., 2000; Gloor et al., 2017).

2.9. Statistical analysis

Further data-handling was done in R (version 3.6.3) with the Rstudio GUI (version 1.1.453). Custom R scripts are available at at https://github.com/thomazbastiaanssen/Tjazi (Bastiaanssen, 2018). Stacked barplots were generated by normalizing counts to 1, generating proportions. Genera that were never detected at a 1% relative abundance or higher were aggregated and defined as rare taxa for the purposes of the stacked barplots. Principal component analysis was performed on centred log-ratio transformed (clr) values using the ALDEx2 library (Fernandes et al., 2014). Number of permutations was always set to 1000. Volatility was measured as distance between before and after the experiment or treatment and was calculated as the Aitchison distance between the two timepoints. Unlike other distance metrics such as Bray-Curtis and Jensen-Shannon divergence, the Aitchison distance takes into account the compositional nature of microbiome datasets (Aitchison et al., 2000). Piphillin was used for functional inference from 16S rRNA gene seqeunce of mouse stool samples in the form of KEGG orthologues (Iwai et al., 2016). Gut-Brain Modules (GBMs) and Gut-Metabolic Modules (GMMs) were calculated using the R version of the Gomixer tool (Valles-Colomer et al., 2019). Differential abundance of both microbes and functional modules were calculated using implementations of the ALDEx2 library. As part of testing for correlations between volatility and metadata, skadi, an implementation of jackknifing and Grubb's test, was used to assess reliability of the data and detect outliers (Bastiaanssen, 2018). Correlation was assessed using Spearmans's rank correlation coefficient in the case of low N or heteroskedacity. In all other cases the Pearson correlation coefficient was used. Normality was assessed using the Shapiro-Wilk test. For normally distributed data, between-group differences were analysed using ANOVA or unpaired two-tailed t-test and Tukey's test for post-hoc analysis. For datasets in which the condition of normality was violated the non-parametric Kruskal-Wallis test was used and post-hoc analysis was done using the Wilcoxon test. A p-value of <0.05 was deemed significant in all cases. To correct for multiple testing in tests involving microbiota or Functional Modules, the Benjamini-Hochberg (BH) post-hoc was performed with a q-value of 0.1 as a cut-off. R scripts are available online on GitHub (https://github.com/thomazbastiaanssen/Volatility).

3. Results

3.1. The gut microbiome is differentially volatile in response to chronic social defeat stress

After filtering, 137 different genera were detected in the mouse microbiome samples (Fig. 2A, B). Differential abundance analysis can be found in the supplementary files at genus (Supplementary Figure S1) and at higher taxonomic levels (Supplementary Figure S2). In the Validation cohort, stressed animals showed more changes in their microbiome compared to control animals. To quantify volatility, which we

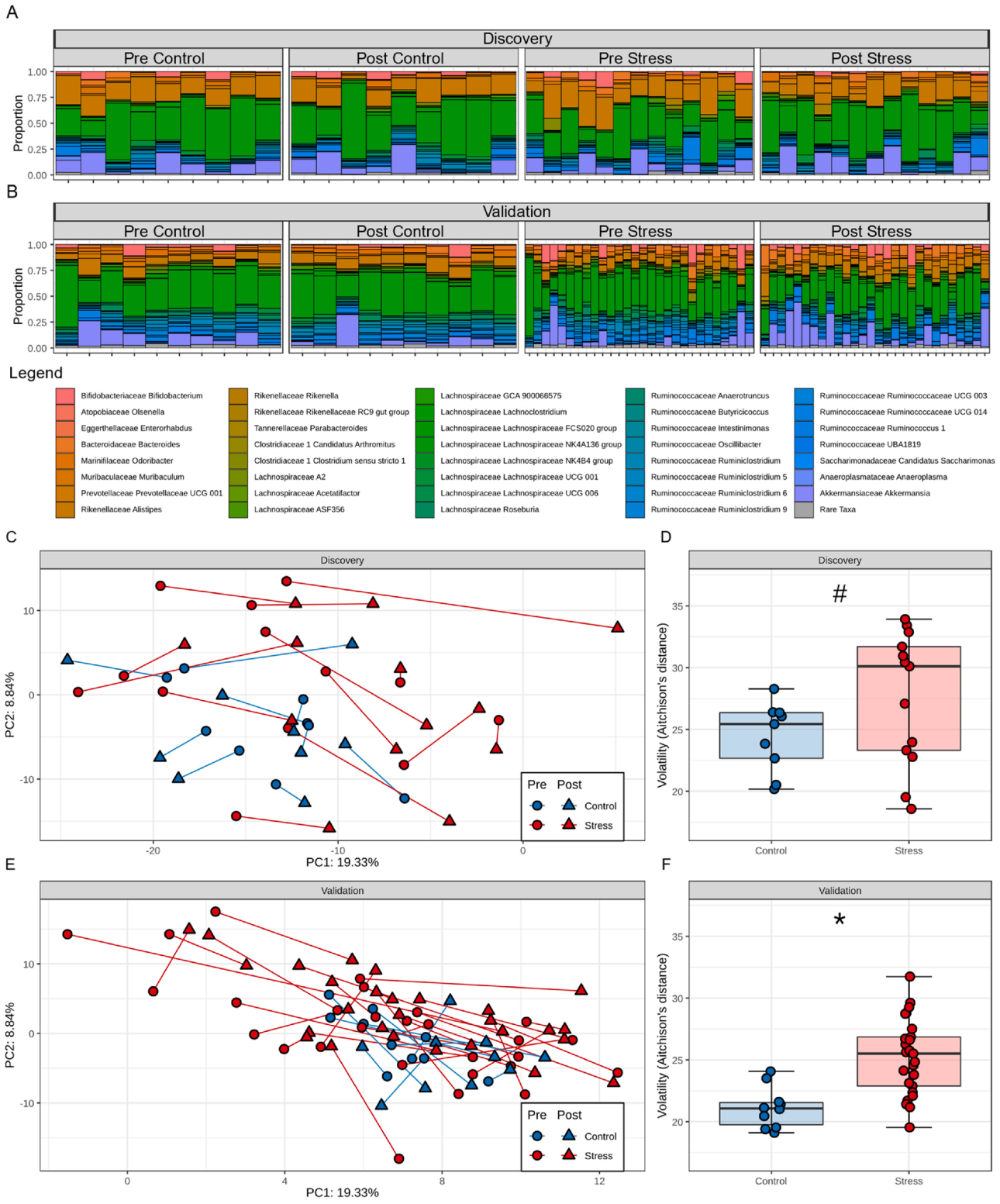


Fig. 2. Microbial volatility is influenced by stress. (A) Stacked barplot showing the proportion of genera based on 16S sequences detected per sample in the Validation cohort and the (B) Discovery cohort. Volatility was defined as the Aitchison distance travelled over the 10-day experiment. (C) PCA showing the microbiome compositions of animals before and after the 10-day period. Lines link the same animal over time, showing the trajectory and distance travelled in time. (D) Aitchison distance travelled is shown on the y-axis; Mann-Whitney $p = 0.093$, $W = 38$, $d = 0.7$. (E) The PCA of the validation cohort and (F) corresponding elevated volatility in stressed mice.; Mann-Whitney $p = 5.06 \times 10^{-5}$, $W = 30$, $d = 1.66$. Discovery cohort: Control N = 9; Stress: N = 13, validation Control cohort: N = 10; Stress: N = 28.

define as the degree of compositional change of the microbial ecosystem over time, we calculated the intra-subject Aitchison distance between the genus-level count tables from the same subject taken before and after the experiment using the clr-transformation (Fig. 2C). Stressed mice showed a trend toward a significantly higher degree of volatility when compared to controls in the Discovery cohort (Fig. 2D). In the Discovery cohort, we found that volatility correlated with several measurements associated with the stress response, including social avoidance behaviour (Fig. 3). In the first cohort, we randomly selected samples for 16S rRNA gene sequencing from the original 27 stressed and 29 control mice (Gururajan et al., 2019). All data regarding the Discovery cohort in this manuscript only refers to the subset of animals of which the faecal microbiome was sequenced. We ran a second larger independent cohort under the same conditions, the Validation cohort, with the intent to verify our results from the smaller Discovery cohort. In the Validation cohort, we again found a higher degree of volatility in stressed animals compared to controls, this time significantly so (Fig. 2E-F).

3.2. Volatility of the gut microbiome is correlated with aspects of the stress response

Pursuing the elevated volatility in stressed animals compared to controls, we found a significant correlation between social avoidance behaviour and volatility. This finding was again replicated in the validation cohort (Fig. 4).

3.3. Volatility is correlated with absolute change in measures of alpha-diversity

As alpha-diversity and beta-diversity are related metrics, we asked whether changes in beta-diversity, volatility, would be related to changes in alpha diversity. We computed alpha-diversity based on the first three hill-numbers; Chao1, Simpson and Shannon and found correlations between these metrics and volatility in both cohorts in the stressed mice, but never in controls (Fig. 5).

3.4. Perceived Stress in Humans

To investigate whether the relation between volatility and stress was observed in humans, we tested for correlation between volatility and stress as measured by the Perceived Stress Scale (PSS) in a cohort of students undergoing academic exams. We found a significant correlation between volatility and PSS during stress, but not under non-stress conditions i.e., before the exam period (Fig. 6). On their own, we detected no differential abundance in terms of genera, Gut-Brain Modules or Gut Metabolic Modules in the human cohort after FDR (Supplementary Tables 6-8).

Additionally, we set out to compare the relationship between blood cortisol and corticosterone and volatility in our human and mouse cohorts, respectively. In humans, we found a correlation between the cortisol awakening response (AUC) and volatility. Analogously, we found a significant positive correlation between evening corticosterone levels in the discovery mouse cohort as well as a trend in the same direction in the validation mouse cohort (Fig. 7) using the Pearson

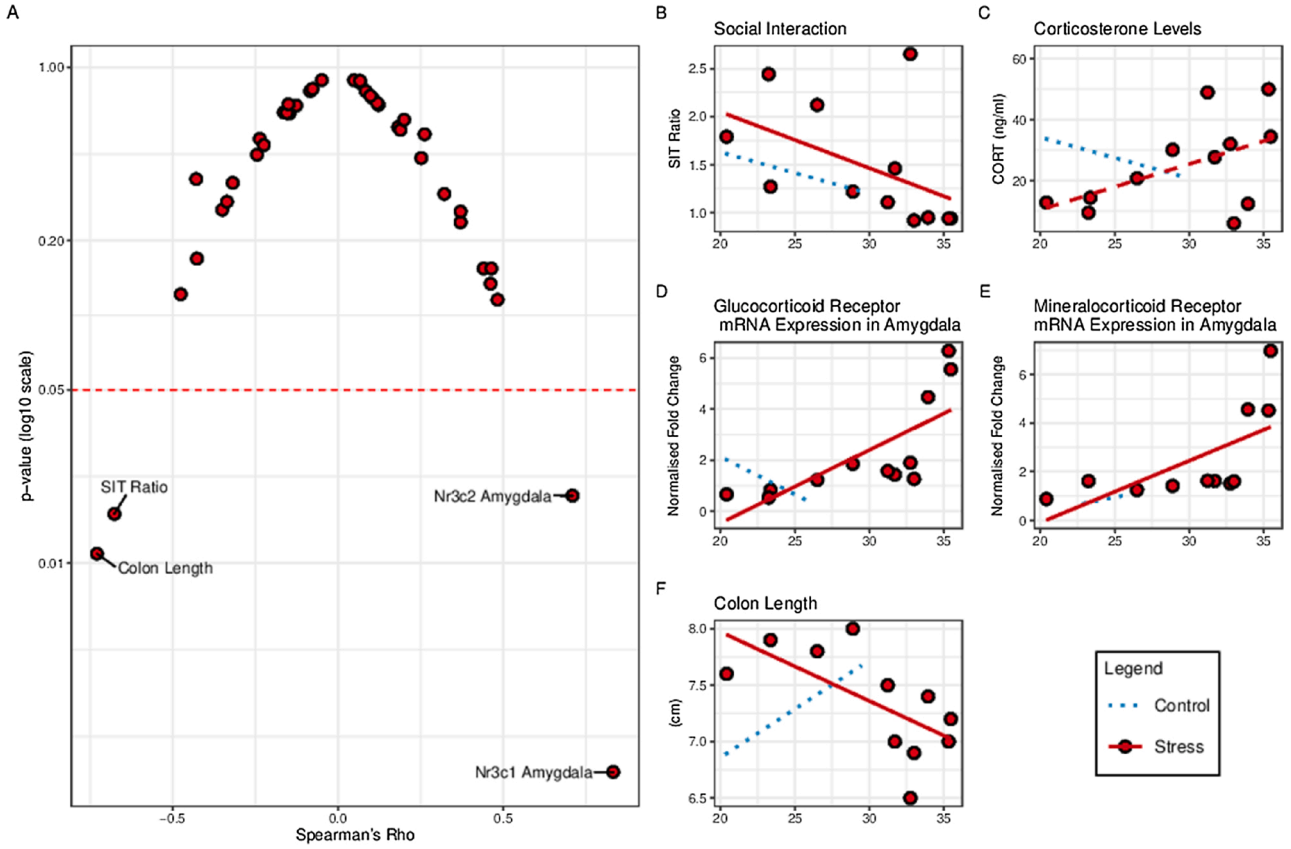


Fig. 3. Volatility correlates with biological measures associated with stress. (A) Inverse volcano plot showing correlations between volatility and all other experimental parameters. Y and x-axis represent p-value on a log10 scale and spearman's rho, respectively. Red dashed line depicts p = 0.05. (B-F) Show individual correlations between all experimental values (y-axis) and volatility (x-axis). Measurements were assessed for reliability using Grubbs' test for outliers and jackknifing. Controls and outliers were left out for correlations (opaque circles). Lines represent the fitted regression line, with a full line indicating a significant correlation, while a dotted line indicates no significance. Spearman: (B); p = 0.016, rho = -0.676 (C); p = 0.154, rho = 0.441 (D, Nr3c1); p = 0.001, rho = 0.832 (E, Nr3c2); p = 0.019, rho = 0.709 (F); p = 0.011, rho = -0.730. Discovery cohort: Control N = 9; Stress: N = 13.

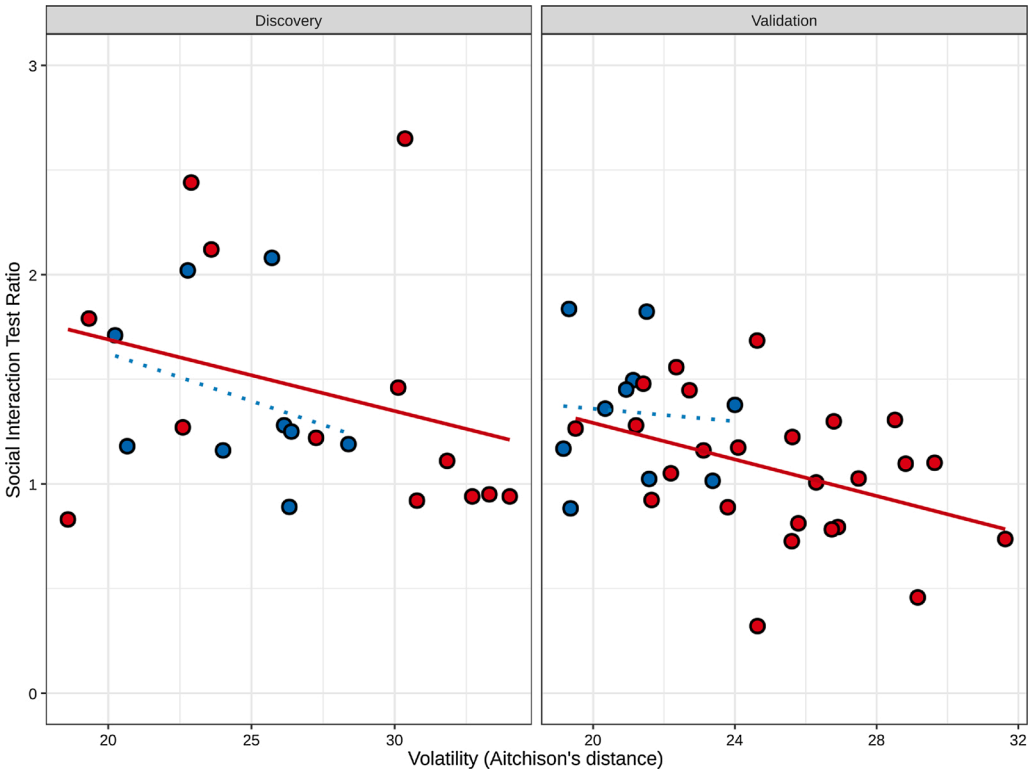


Fig. 4. Microbial volatility is negatively correlated with Social Interaction Ratio after Social Defeat Stress. The x-axis shows volatility as defined by Aitchison distance moved over 10 days, while the y-axis shows the Social Interaction Ratio as defined by the ratio of time spent in proximity to an unfamiliar CD-1 mouse and time spent in the same area without the second mouse present. Red points show stressed mice, while blue points show controls. Lines represent the fitted regression line, with a full line indicating a significant correlation, while a dotted line indicates no significance. Spearman: In the Discovery cohort (Left); p = 0.017, rho = -0.68. In the Validation cohort (Right); p = 0.045, rho = -0.41. Discovery cohort: N = 8; stress: N = 13, validation cohort: N = 10; Stress: N = 28.

correlation coefficient, even though we did not find significance in the validation cohort using the Spearman correlation coefficient, likely due to low N (Fig. 3C).

3.5. *Comparing Microbiome response to stress across cohorts*

Finally, we set out to investigate whether other types of changes could be replicated between cohorts. We assessed differential abundance on the genus level in both discovery and validation cohorts as well as differential abundance on the level of Gut-Brain Modules (GBMs) and Gut-Metabolic Modules (GMMs). These modules represent functional pathways curated from literature that have been reported to take place in the microbiome and are involved in either gut-brain communication or in microbiome metabolism, respectively (Valles-Colomer et al., 2019). In order to compare the responses between the two cohorts, the effect sizes representing the change per microbiome feature were extracted and those modules that were present in both cohorts were tested for correlation. In the theoretical case of perfect agreement between cohorts, the rho for the correlation of the effect sizes would be 1. We found no correlation on the genus level in any animals, but in the stressed animals we found a strong positive correlation in the effect sizes on both the GBM and GMM level (Fig. 8). The same procedure was then carried out comparing the functional changes in the mouse cohorts to those in the human cohort. The mouse cohorts were aggregated in order to promote interpretability (Fig. 9). We observed significant correlations between the responses to chronic stress for both GBM and GMM across human and mouse host species. In particular, in terms of effect sizes, GABA synthesis and isovaleric acid synthesis seemed to increase the most in both host species in terms of GBMs, while mucin degradation increased the most in terms of GMMs. Complete differential abundance features results can be found in the supplementary files in terms of genera (Supplementary Table 3) Gut-Brain Modules (Supplementary Table 4) and Gut-Metabolic Modules (Supplementary Table 5). A heatmap visual representation of these differentially abundant features can be found as well (Supplementary Fig. 1).

4. Discussion

Microbiome volatility is a relatively underutilized concept in microbiome ecology. With regard to stress it has not been explored previously. One exception is in the context of irritable bowel syndrome (IBS) (Halfvarson et al., 2017), a condition which has been linked to physical and psychosocial stress exposure (Mayer et al., 2001) or Inflammatory Bowel Disease (IBD), which has also been linked to stress and anxiety (Mawdsley and Rampton, 2005), both of which found display more volatility (though it was not referred to as such) in patients compared with healthy controls (Clooney et al., 2020; Ryan et al., 2020). In this study, we further investigated the concept of volatility and, for what is to our knowledge the first time, report its potential influence on stress-related central and peripheral phenotypes.

We firstly showed that mice which had higher values in biological measures commonly associated with stress, such as changes in corticosterone levels also showed an increased volatility. Secondly, we observed a significant negative correlation between volatility and social behaviour. Notably, this correlation was found in both the discovery and validation cohorts. The implication is that severity of the stressor is related to degree of volatility, indicating volatility is related to stress susceptibility and resilience. Clearly, some stressed animals showed a higher degree of volatility than others. There are two possible explanations for this observation. The first is that volatility is determined by the microbiome, which would imply that a more volatile microbiome is a marker of stress susceptibility. Conversely, a more stable microbiome would then be a marker of stress resilience. Second, an elevated volatility after stress could be the result of a more severe reaction to stress. Indeed, exposure to stress has often been associated with changes in the microbiome (Bharwani et al., 2016; Langgartner et al., 2018). Stress is known to change host physiology and behaviour including diet, both of which are known to impact the microbiome (David et al., 2014; Lucking et al., 2018; O'Connor et al., 2020; O'Connor et al., 2019). This makes it seem likely that volatility is determined at least to a degree by stress. However, recently more attention has been brought upon the temporal dynamics including stability and drift in the microbiome, viewing it as

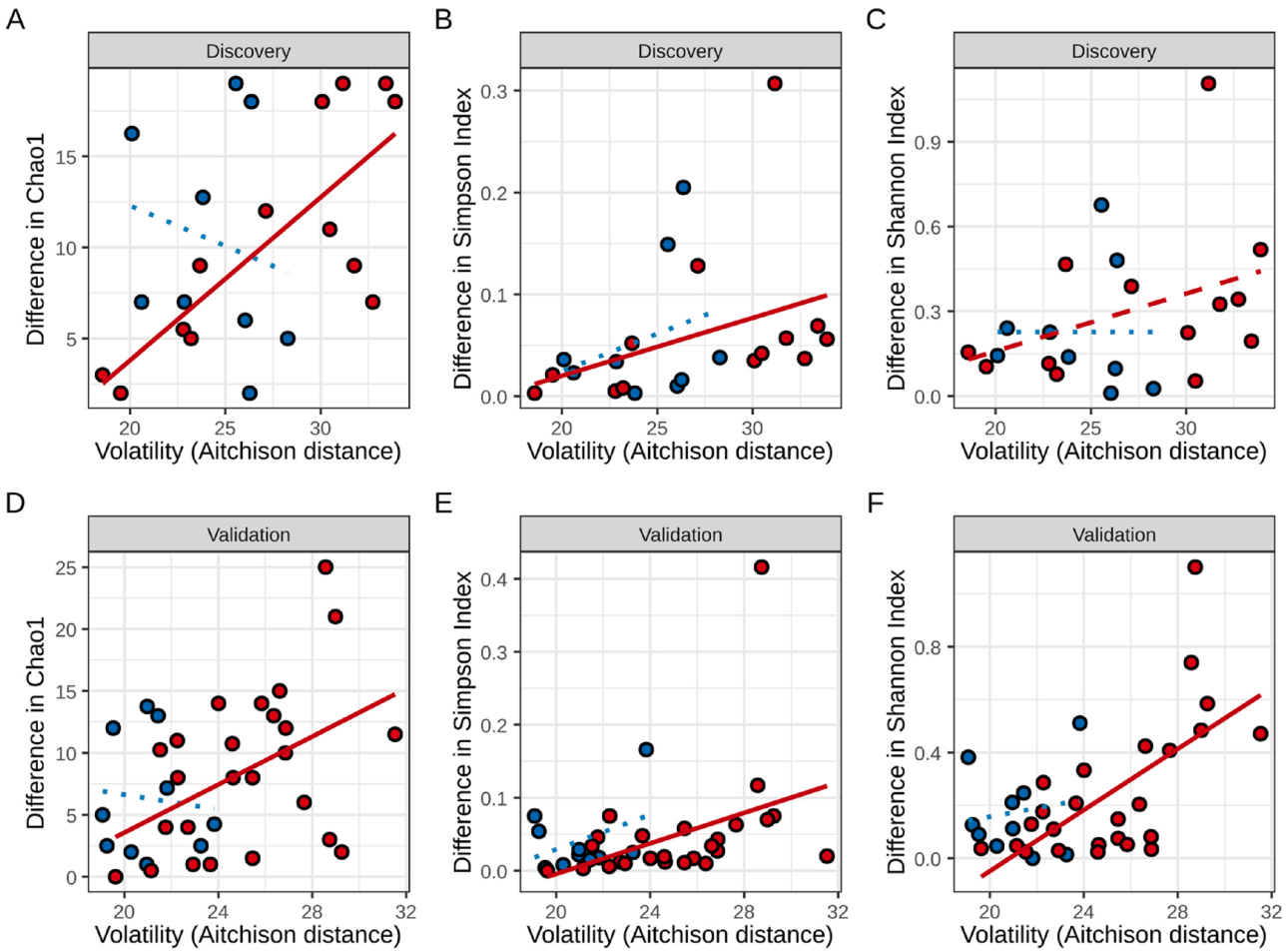


Fig. 5. Microbial volatility is positively correlated with absolute changes in Alpha Diversity indices after Social Defeat Stress. The x-axis shows volatility as defined by Aitchison distance moved over 10 days, while the y-axis shows the absolute difference in Chao1 (A,D), Simpson Index (B,E) and Shannon Index (C,F) on a genus level. A-C relates to the discovery cohort, whereas D-F corresponds to the Validation cohort. Red points show stressed mice, while blue points show controls. Lines represent the fitted regression line, with a full line indicating a significant correlation, a dashed line showing a trend ($0.05 < p < 0.1$) while a dotted line indicates no significance. Spearman: (A); $p = 0.004$, rho = 0.739 (B); $p = 0.014$, rho = 0.687 (C); $p = 0.10$, rho = 0.495 (D); $p = 0.024$, rho = 0.43 (E); $p = 0.003$, rho = 0.55 (F); $p = 0.002$, rho = 0.55. Discovery cohort: Control N = 9; Stress: N = 13, validation Control cohort: N = 10; Stress: N = 28.

an ecological system (Kenney et al., 2020). Previous research reported that both external and microbiome composition itself influence microbiome stability (Gibbons et al., 2017). Determining causality in non-linear systems like the microbiome brings its own set of challenges. For instance, different factors often cannot be separated as they together represent the system (Sugihara et al., 2012). Taken together, it is entirely possible that stress and volatility could be in a positive feedback loop with each influencing and potentially exacerbating the other. Research incorporating more frequent and numerous timepoints could shed light on this question. A similar approach was recently employed to great effect to characterise the interaction between diet and the microbiome (Johnson et al., 2019). There, diet was shown to partially explain changes in microbiome composition over very short timeframes.

While inconclusive, we initially sought to identify features in the baseline microbiome that could explain the degree of volatility after stress (data not shown), however, such differences in baseline did not hold up in the validation cohort or in the human study. Here, we were unable to find predictor features in the baseline microbiome that were generalizable over all three cohorts. In future studies, the volatility at baseline as opposed to single point measures in the current study could be measured to address this question. Specifically, one may hypothesize that hosts with the most volatile microbiomes during neutral conditions could be the most susceptible to stress and that low volatility is a predictor of stress resilience. Additionally, future studies are needed to examine the correlation between volatility and other phenotypes of relevance following chronic stress.

The findings of this study have potential translational implications in understanding volatility in the context of human health. Indeed, the fact that we observed a correlation between self-reported stress during academic exams and volatility strengthens the notion that volatility is closely associated to stress and stress resilience. For example, one could consider volatility in the context of microbial-based interventions to treat stress-induced psychopathologies, formulations designed to stabilise the microbiome could be administered over a period of time to improve response. Alternatively, given that psychotropics are themselves known to influence the microbiome (Cussotto et al., 2019), we speculate that pre-treatment with psychobiotics which introduce specific keystone species into the microbiome may make it more receptive to the therapeutic effects of antidepressants or anxiolytics. This latter approach could be relevant especially for patients who are resistant to treatment using conventional approaches. This also opens the door to keystone species, species that when absent will destabilize the gut ecosystem, in psychobiotic formulations. On its own, it is unclear what the impact a more volatile microbiome could be on host health, if any. One could hypothesise that volatility destabilizes the microbiome resulting in an increased susceptibility for bacterial taxa to colonize. We did not find evidence of this in this study, but this might be due to the sanitary housing conditions of the animals and that mice are

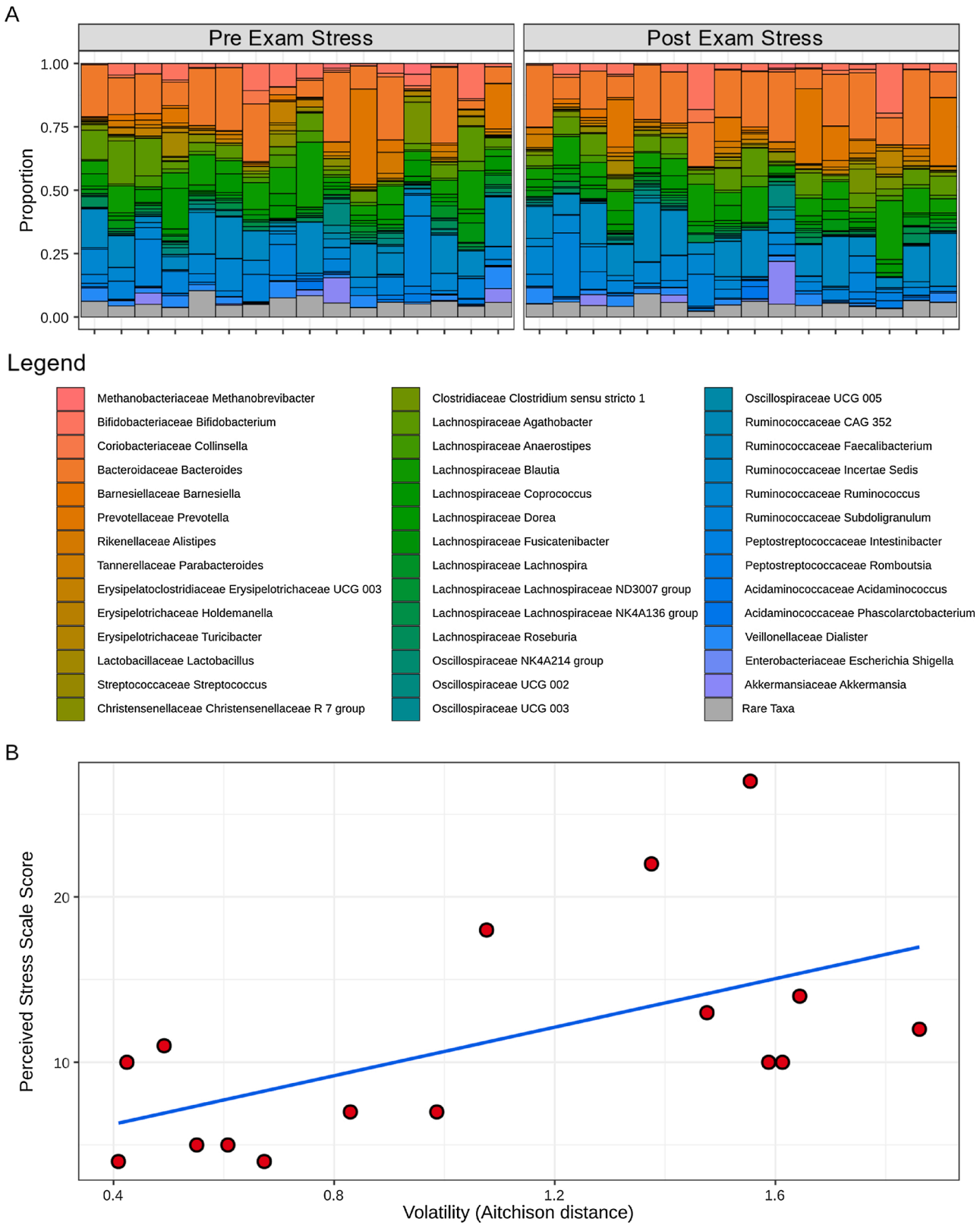


Fig. 6. ***Microbial volatility is correlated with Perceived Stress during academic exam stress.*** *(A) Stacked barplot showing the proportion of genera based on 16S sequences detected per sample. (B) The x-axis shows volatility as defined by Aitchison distance while the y-axis shows the Perceived Stress Scale Score during academic exam stress. Line represents the fitted regression line indicating a significant correlation. N = 16, Spearman: p = 0.028, rho = 0.55.*

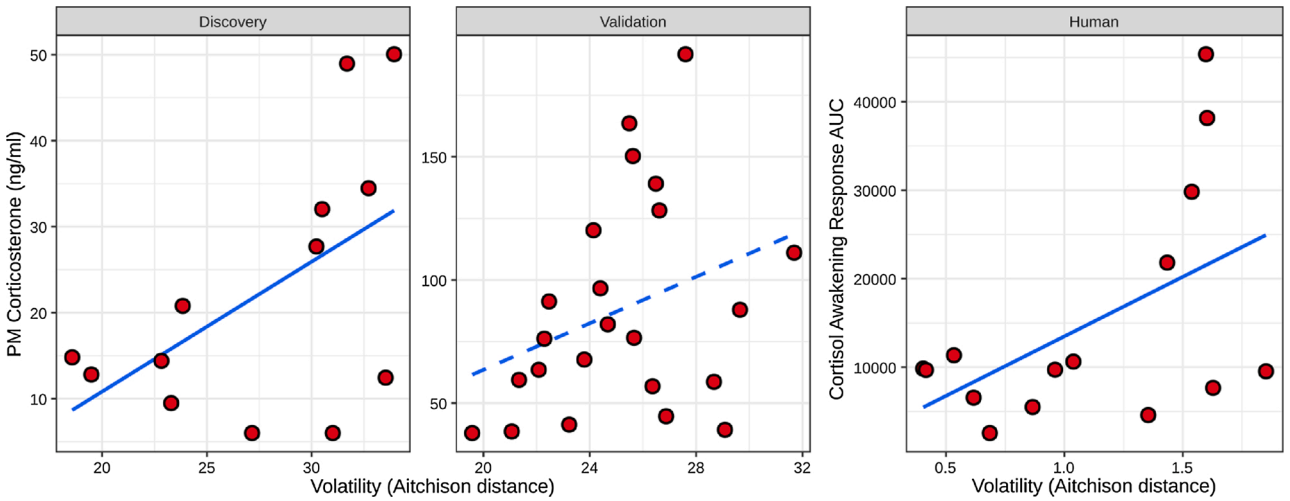


Fig. 7. Microbial volatility is positively correlated with Cortisol and Corticosterone after chronic stress in humans and mice, respectively. The x-axis shows volatility as defined by Aitchison distance while the y-axis shows the evening corticosterone levels in the two left-most figures and the Cortisol Awakening Response in the rightmost figure. Line represents the fitted regression line, with a full line indicating a significant correlation and a dashed line indicating a statistical trend. Pearson: Discovery: p = 0.0293, rho = 0.537; Validation: p = 0.063, rho = 0.327; Human: p = 0.024, rho = 0.517. Discovery cohort: Control: N = 8; Stress: N = 13, validation cohort: N = 10; Stress: N = 28, human cohort: N = 16.

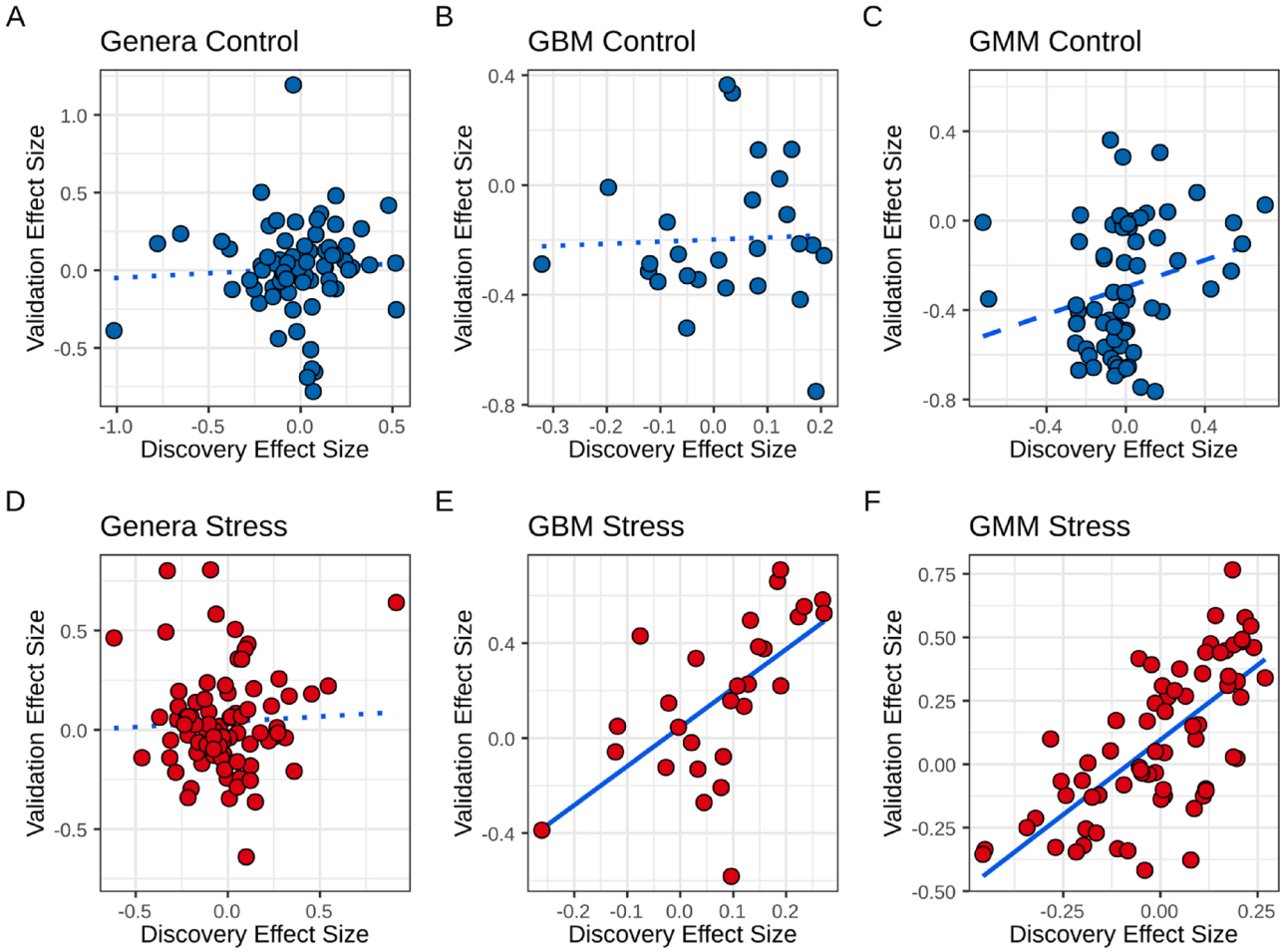


Fig. 8. Microbiome responds to stress similarly on a functional level but not on a taxonomical level. The x-axis shows the effect size per feature of the Discovery cohort, while the y-axis shows the effect size fer feature of the Validation cohort. Every dot represents one microbial feature. The top (blue; A-C) row shows the comparisons from the Controls, while the bottom (red; D-F) shows the comparisons for the Stressed animals. Lines represent the fitted regression line, with a full line indicating a significant correlation, a dashed line showing a trend ($0.05 < p < 0.1$) while a dotted line indicates no significance. Pearson: (A); p = 0.657 rho = 0.052 (B); p = 0.844, rho = 0.04 (C); p = 0.051, rho = 0.243 (D); p = 0.674, rho = 0.04 (E); p = 0.0004, rho = 0.625 (F); $p = 1.9 \times 10^{-11}$, rho = 0.694.

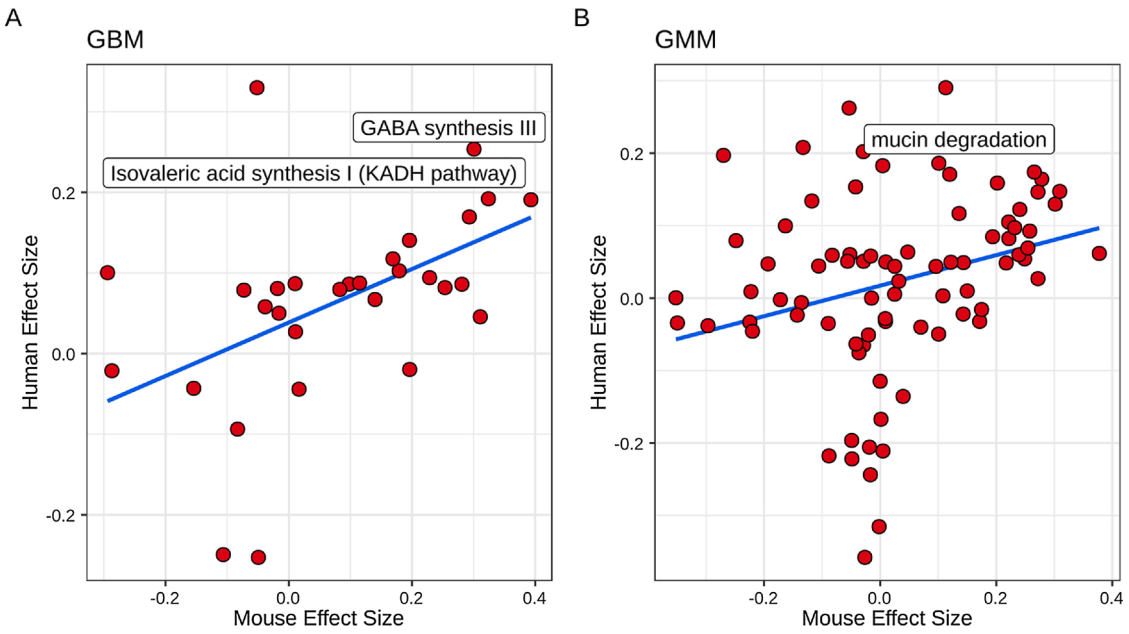


Fig. 9. Microbiome responds to stress similarly on a functional level across mice and humans. The x-axis shows the effect size per feature of the Mouse cohort, while the y-axis shows the effect size fer feature of the Human cohort. The left (A) figure shows Gut-Brain modules while the right (B) figure shows Gut-Metabolic Modules. Every dot is one microbial feature. Labels show the module names that had an absolute effect size higher than 0.2 in both mice and humans. Lines represent the fitted regression line, with a full line indicating a significant correlation. Pearson: (A); p = 0.005 rho = 0.494 (B); p = 0.011, rho = 0.279.

coprophagic. Indeed, fecal microbiota transplantation, representing a high-alpha-diversity pool, has been shown to expedite colonization rate over natural recovery (Suez et al., 2018). Moreover, the microbiome has been shown to regulate microbiome ecosystem stability (Gibbons et al., 2017). More research is warranted to test this hypothesis.

We also found consistent changes across cohorts and even between mice and humans in the microbiome after chronic stress. We did not, however, find such agreement at a taxonomic level. This is likely due to the differences in baseline microbiome between the two cohorts and the humans. Indeed, in humans, it is well-known that interpersonal variability is much lower on the functional level than on the taxonomical level (Human Microbiome Project Consortium, 2012; Mehta et al., 2018). From our findings we extrapolate that in the context of stress, while the taxonomical changes of the microbiome seem to be cohort-dependent and ultimately baseline microbiome dependent, there is a strong agreement in how the functional microbiome changes after a stressor. A stress response in the microbiome that seems invariant of the baseline condition could indicate some sort of adaptive stress response, either on the level of the microbiome or on the host level. Per definition, the GMMs and especially the GBMs have functional implications for host health. In a recent study, GBMs were shown to be influenced by diet (Butler et al., 2020; Valles-Colomer et al., 2019). Together with the finding that stress influences these modules in a specific manner, this opens up the door for psychobiotics that specifically aim to control the levels of specific modules that are known to be altered by stress. Notably, GABA synthesis was altered in both our human and mouse cohorts. The GABAergic system has been previously shown to be modulated by the microbiome in the context of the stress response (Bravo et al., 2011) and GABA-modulating bacteria have been implicated in stress-related disorders such as depression in humans (Strandwitz et al., 2019). In addition, the synthesis pathway of the short-chain fatty acids propionate and isovalerate were inferred to be upregulated after stress. These metabolites have been shown to influence stress-resilience (van de Wouw et al., 2018). Similarly, tryptophan metabolism and quinolinic acid synthesis was found to be increased after stress and have been implicated in mental health (Cervenka et al., 2017; Schwarcz et al., 2012). Alterations in menaquinone (vitamin K2) synthesis after stress were also found. Menaquinone has anti-oxidant properties and has been reported to confer neuroprotective effects (Farhadi Moghadam and Fereidoni, 2020). Notably, most of the significant changes after stress had a positive effect size, indicating consistent gains of functions occurring. This together with the correlations we reported in the changes in GBMs and GMMs following stress supports the notion that there is a directed response to stress in the microbiome. Indeed, there has been speculation that some alterations in the microbiome due to a stressor could actually be adaptations to protect the host (Walter et al., 2020). These alterations should be pursued in future research.

To conclude, we propose that an analysis of volatility should be considered in all future longitudinal microbiome research projects. Given the novelty of this concept, we make some basic recommendations as to how examine this variable. The approach to calculating volatility presented here relies on Aitchison distance. This metric was selected because it was specifically designed to deal with compositional data, such as the microbiome (Aitchison et al., 2000; Gloor et al., 2017). While other metrics for beta-diversity do exist (Bokulich et al., 2018), Aitchison distance has the added benefit of satisfying the criteria for being a Euclidean distance, making comparisons between two distances within the same analysis possible. Other popular metrics like Bray-Curtis or Unifrac do not have this property, but rather give relative distance on a scale from zero to one, making them less suitable for the purpose of assessing volatility. We speculate that further convergence of high-dimensional mathematics, microbiology and genetics will lead to newer algorithms which prove to be more useful and easier to use. For instance, the *phILR* beta-diversity metric combines the taxonomic framework of Unifrac with the compositionally appropriate Ilr-transformation and should be explored in further volatility research (Silverman et al., 2017). Lastly, in this study, volatility was calculated by assessing the distance 'travelled' between two points over time. Future studies should consider collecting samples over multiple time points (e.g. during stress exposure) to produce higher-dimensional geometric shapes in microbiome-space which could lead to more nuanced insights into the role of the microbiome as a mediator of the stress response.

Funding

This research was funded by a Marie-Sklowdowska-Curie Actions Individual Fellowship to AG from the European Research Council (Grant Number 704995), MyNewGut (human study; Grant number 613979) and conducted in the APC Microbiome Institute, which is funded by Science Foundation Ireland (SFI; grant nos. SFI/12/RC/2273). TGD and JFC are also supported by the Irish Health Research Board, the Dept. of Agriculture, Food and the Marine and Enterprise Ireland. TGD and JFC are the principal investigators in the APC Microbiome Institute, University College Cork. The authors declare that they have no competing interests. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The APC Microbiome Institute has conducted research funded by many pharmaceutical and food companies. TGD has been an invited speaker at meetings organized by Servier, Lundbeck, Janssen and AstraZeneca and has received research funding from Mead Johnson, Cremo, Wellness, Nutricia and 4D Pharma. JFC has been an invited speaker at meetings organized by Mead Johnson, Yakult, Alkermes and Janssen and has received research funding from Mead Johnson, Cremo, Suntory Wellness, Nutricia and 4D Pharma.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. In particular, mouse microbiome data (Supplementary Table 2) and stress-associated measurements (Supplementary Table 1) from the validation cohort.

Author contributions

TFSB, AG and JFC designed the study. AG and MVW carried out all stress procedures and behavioural experiments. CML carried out the perceived stress experiments in human subjects. AG, MVW, NLR, GM, CML and JL carried out the behavioural and physiological analyses. NCW, ABM and FF performed 16S-RNA sequencing. TFSB carried out the microbiome and bioinformatics analyses. TFSB and AG analysed the data and co-wrote the manuscript. CS, MC, TGD and JFC provided supervision. All authors contributed to the interpretation of the data, critically revised the manuscript and read and approved the final version before submission.

Ethics approval and consent to participate

All animal experiments were conducted conducted in accordance with European Directive 86/609/EEC, Recommendation 2007/526/65/EC, and approved by the Health Products Regulatory Authority (AE19130-P031) as well as the Animal Experimentation Ethics Committee of University College Cork. All efforts were made to minimize animal suffering and to reduce the number of animals used.

The human study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (study number APC080) and conducted in accordance with the ICH Guidelines on Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from all participants at the screening visit, before any study procedures were conducted. Participants were free to withdraw from the study at any time.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

We wish to thank Dr Kieran Rea, Dr Anna Golubeva and Dr Kenneth O'Riordan for helpful comments on the manuscript, Mr Patrick Fitzgerald and Ms Collette Manley for technical assistance and animal husbandry and the Teagasc Sequencing Facility (Dr Paul Cotter, Dr Fiona Crispie, Dr. Conall Strain and Ms Laura Finnegan) for providing access to the sequencing platform. We thank Anne Marie Cusack for assistance in the human study visits and collection of biological samples, and Susan Rafferty-McArdle for her help in the experimental design of the human study. We thank Danielle Dorland and Sara Firuzeh Hojabri for assistance with biological sample processing and data input. We are thankful to the participants of the academic stress study for their contribution.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.105047.

References

Aitchison, J., Barceló-Vidal, C., Martín-Fernández, J.A., Pawlowsky-Glahn, V., 2000. Logratio Analysis and Compositional Distance. Math Geol 32, 271–275.

Allen, A.P., Hutch, W., Borre, Y.E., Kennedy, P.J., Temko, A., Boylan, G., Murphy, E., Cryan, J.F., Dinan, T.G., Clarke, G., 2016. Bifidobacterium longum 1714 as a translational psychobiotic: Modulation of stress, electrophysiology and neurocognition in healthy volunteers. Translational Psychiatry 6, e939.

Bailey, M.T., Dowd, S.E., Galley, J.D., Hufnagle, A.R., Allen, R.G., Lyte, M., 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav Immun 25, 397–407.

Bastiaanssen, T.F.S., 2018. Tjazi: Microbiome Oriented Compositional Data Toolkit, 0.0.0.1 ed. GitHub.

Bastiaanssen, T.F.S., Cowan, C.S.M., Claesson, M.J., Dinan, T.G., Cryan, J.F., 2018. Making Sense of … the Microbiome in Psychiatry. International Journal of Neuropsychopharmacology pyy067-pyy067.

Bastiaanssen, T.F.S., Cussotto, S., Claesson, M.J., Clarke, G., Dinan, T.G., Cryan, J.F., 2020. Gutted! Unraveling the Role of the Microbiome in Major Depressive Disorder. Harv Rev Psychiatry 28, 26–39.

Bharwani, A., Mian, M.F., Foster, J.A., Surette, M.G., Bienenstock, J., Forsythe, P., 2016. Structural & functional consequences of chronic psychosocial stress on the microbiome & host. Psychoneuroendocrinology 63, 217–227.

Bharwani, A., Mian, M.F., Surette, M.G., Bienenstock, J., Forsythe, P., 2017. Oral treatment with Lactobacillus rhamnosus attenuates behavioural deficits and immune changes in chronic social stress. BMC Med 15, 7.

Bokulich, N.A., Dillon, M.R., Zhang, Y., Rideout, J.R., Bolyen, E., Li, H., Albert, P.S., Caporaso, J.G., 2018. q2-longitudinal: longitudinal and paired-sample analyses of microbiome data. MSystems, p. 3.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., Cryan, J.F., 2011. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America 108, 16050–16055.

Burokas, A., Arboleya, S., Moloney, R.D., Peterson, V.L., Murphy, K., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2017. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry 82, 472–487.

Butler, M.I., Bastiaanssen, T.F.S., Long-Smith, C., Berding, K., Morkl, S., Cusack, A.M., Strain, C., Busca, K., Porteous-Allen, P., Claesson, M.J., Stanton, C., Cryan, J.F., Allen, D., Dinan, T.G., 2020. Recipe for a Healthy Gut: Intake of Unpasteurised Milk Is Associated with Increased Lactobacillus Abundance in the Human Gut Microbiome. Nutrients 12.

Caporaso, J.G., Lauber, C.L., Costello, E.K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J., Knights, D., Gajer, P., Ravel, J., Fierer, N., Gordon, J.I., Knight, R., 2011. Moving pictures of the human microbiome. Genome Biol 12. R50-R50.

Cervenka, I., Agudelo, L.Z., Ruas, J.L., 2017. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science 357.

Claesson, M.J., Cusack, S., O'Sullivan, O., Greene-Diniz, R., de Weerd, H., Flannery, E., Marchesi, J.R., Falush, D., Dinan, T., Fitzgerald, G., Stanton, C., van Sinderen, D., O'Connor, M., Harnedy, N., O'Connor, K., Henry, C., O'Mahony, D., Fitzgerald, A.P., Shanahan, F., Twomey, C., Hill, C., Ross, R.P., O'Toole, P.W., 2011. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci USA 108, 4586–4591.

Clooney, A.G., Eckenberger, J., Laserna-Mendieta, E., Sexton, K.A., Bernstein, M.T., Vagianos, K., Sargent, M., Ryan, F.J., Moran, C., Sheehan, D., 2020. Ranking microbiome variance in inflammatory bowel disease: a large longitudinal intercontinental study. Gut.

Consortium, H.M.P., 2012. Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214.

Cruz-Pereira, J.S., Rea, K., Nolan, Y.M., O'Leary, O.F., Dinan, T.G., Cryan, J.F., 2020. Depression's unholy trinity: dysregulated stress, immunity, and the microbiome. Annual review of psychology 71.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, Kiran V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K. E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Pereira, J., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons, S.E., Hyland, N., Clarke, G., Dinan, T.G., 2019. The Microbiota-Gut-Brain Axis. Physiological Reviews.

Cussotto, S., Clarke, G., Dinan, T.G., Cryan, J.F., 2019. Psychotropics and the Microbiome: a Chamber of Secrets. Psychopharmacology 1–22.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559–563.

Dinan, T.G., Cryan, J.F., 2012. Regulation of the stress response by the gut microbiota: Implications for psychoneuroendocrinology. Psychoneuroendocrinology 37, 1369–1378.

Donoso, F., Egerton, S., Bastiaanssen, T.F., Fitzgerald, P., Gite, S., Fouhy, F., Ross, R.P., Stanton, C., Dinan, T.G., Cryan, J.F., 2020. Polyphenols selectively reverse early-life stress-induced behavioural, neurochemical and microbiota changes in the rat. Psychoneuroendocrinology, 104673.

Farhadi Moghadam, B., Fereidoni, M., 2020. Neuroprotective effect of menaquinone-4 (MK-4) on transient global cerebral ischemia/reperfusion injury in rat. Plos one 15, e0229769.

Fernandes, A.D., Reid, J.N., Macklaim, J.M., McMurrough, T.A., Edgell, D.R., Gloor, G.B., 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2, 15.

Foster, J.A., Rinaman, L., Cryan, J.F., 2017. Stress & the gut-brain axis: Regulation by the microbiome. Neurobiol Stress 7, 124–136.

Gautam, A., Kumar, R., Chakraborty, N., Muhie, S., Hoke, A., Hammamieh, R., Jett, M., 2018. Altered fecal microbiota composition in all male aggressor-exposed rodent model simulating features of post-traumatic stress disorder. J Neurosci Res 96, 1311–1323.

Gibbons, S.M., Kearney, S.M., Smillie, C.S., Alm, E.J., 2017. Two dynamic regimes in the human gut microbiome. PLoS computational biology 13, e1005364.

Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome datasets are compositional: and this is not optional. Front Microbiol 8, 2224.

Goodrich, J.K., Di Rienzi, S.C., Poole, A.C., Koren, O., Walters, W.A., Caporaso, J.G., Knight, R., Ley, R.E., 2014. Conducting a microbiome study. Cell 158, 250–262.

Gururajan, A., van de Wouw, M., Boehme, M., Becker, T., O'Connor, R., Bastiaanssen, T. F.S., Moloney, G.M., Lyte, J.M., Ventura Silva, A.P., Merckx, B., Dinan, T.G., Cryan, J.F., 2019. Resilience to chronic stress is associated with specific neurobiological, neuroendocrine and immune responses. Brain, Behavior, and Immunity 80, 583–594.

Halfvarson, J., Brislawn, C.J., Lamendella, R., Vázquez-Baeza, Y., Walters, W.A., Bramer, L.M., D'Amato, M., Bonfiglio, F., McDonald, D., Gonzalez, A., McClure, E.E., Dunklebarger, M.F., Knight, R., Jansson, J.K., 2017. Dynamics of the human gut microbiome in inflammatory bowel disease. Nat Microbiol 2, 17004-17004.

Hemmings, S.M.J., Malan-Müller, S., van den Heuvel, L.L., Demmitt, B.A., Stanislawski, M.A., Smith, D.G., Bohr, A.D., Stamper, C.E., Hyde, E.R., Morton, J.T., Marotz, C.A., Siebler, P.H., Braspenning, M.I., Van Criekinge, W., Hoisington, A.J., Brenner, L.A., Postolache, T.T., McQueen, M.B., Krauter, K.S., Knight, R., Seedat, S., Lowry, C.A., 2017. The microbiome in posttraumatic stress disorder and trauma-exposed controls: An exploratory study. Psychosomatic Medicine 79, 936–946.

Iwai, S., Weinmaier, T., Schmidt, B.L., Albertson, D.G., Poloso, N.J., Dabbagh, K., DeSantis, T.Z., 2016. Piphillin: improved prediction of metagenomic content by direct inference from human microbiomes. PloS one 11, e0166104.

Jašarević, E., Howard, C.D., Misic, A.M., Beiting, D.P., Bale, T.L., 2017. Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. Scientific Reports 7, 44182.

Johnson, A.J., Vangay, P., Al-Ghalith, G.A., Hillmann, B.M., Ward, T.L., Shields-Cutler, R.R., Kim, A.D., Shmagel, A.K., Syed, A.N., Students, P.M.C., 2019. Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans. Cell host & microbe 25 (789-802), e785.

Kenney, T., Gao, J., Gu, H., 2020. Application of OU processes to modelling temporal dynamics of the human microbiome, and calculating optimal sampling schemes.

Kuti, D., Winkler, Z., Horváth, K., Juhász, B., Paholcsek, M., Stágel, A., Gulyás, G., Czeglédi, L., Ferenczi, S., Kovács, K.J., 2020. Gastrointestinal (non-systemic) antibiotic rifaximin differentially affects chronic stress-induced changes in colon microbiome and gut permeability without effect on behavior. Brain, Behavior, and Immunity 84, 218–228.

Langgartner, D., Vaihinger, C.A., Haffner-Luntzer, M., Kunze, J.F., Weiss, A.-L.J., Foertsch, S., Bergdolt, S., Ignatius, A., Reber, S.O., 2018. The role of the intestinal microbiome in chronic psychosocial stress-induced pathologies in male mice. Frontiers in behavioral neuroscience 12, 252.

Lax, S., Hampton-Marcell, J.T., Gibbons, S.M., Colares, G.B., Smith, D., Eisen, J.A., Gilbert, J.A., 2015. Forensic analysis of the microbiome of phones and shoes. Microbiome 3, 1–8.

Lucking, E.F., O'Connor, K.M., Strain, C.R., Fouhy, F., Bastiaanssen, T.F., Burns, D.P., Golubeva, A.V., Stanton, C., Clarke, G., Cryan, J.F., 2018. Chronic intermittent hypoxia disrupts cardiorespiratory homeostasis and gut microbiota composition in adult male guinea-pigs. EBioMedicine 38, 191–205.

Marin, I.A., Goertz, J.E., Ren, T., Rich, S.S., Onengut-Gumuscu, S., Farber, E., Wu, M., Overall, C.C., Kipnis, J., Gaultier, A., 2017. Microbiota alteration is associated with the development of stress-induced despair behavior. Sci Rep 7, 43859.

Mawdsley, J., Rampton, D., 2005. Psychological stress in IBD: new insights into pathogenic and therapeutic implications. Gut 54, 1481–1491.

Mayer, E.A., Naliboff, B.D., Chang, L., Coutinho, S.V., 2001. V. Stress and irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 280, G519–G524.

Mehta, R.S., Abu-Ali, G.S., Drew, D.A., Lloyd-Price, J., Subramanian, A., Lochhead, P., Joshi, A.D., Ivey, K.L., Khalili, H., Brown, G.T., DuLong, C., Song, M., Nguyen, L.H., Mallick, H., Rimm, E.B., Izard, J., Huttenhower, C., Chan, A.T., 2018. Stability of the human faecal microbiome in a cohort of adult men. Nat Microbiol 3, 347–355.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., Bisson, J.F., Rougeot, C., Pichelin, M., Cazaubiel, M., Cazaubiel, J.M., 2011. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. British Journal of Nutrition 105, 755–764.

Moloney, G., Long-Smith, C.M., Murphy, A., Dorland, D., Firuzeh Hojabri, S., Olavarria Ramirez, L., Campos Marin, D., Bastiaanssen, T.F.S., Cusack, A.-M., Berding, K., Fouhy, F., Allen, A., Stanton, C., Clarke, G., Dinan, T.G., Cryan, J.F., 2020. Submitted. Improvements in sleep indices during exam stress due to consumption of a Bifidobacterium Longum.. Brain, Behavior. and Immunity - Health.

Morais, L.H., Golubeva, A.V., Moloney, G.M., Moya-Pérez, A., Ventura-Silva, A.P., Arboleya, S., Bastiaanssen, T.F., O'Sullivan, O., Rea, K., Borre, Y., 2020. Enduring Behavioral Effects Induced by Birth by Caesarean Section in the Mouse. Current Biology.

O'Connor, K.M., Lucking, E.F., Bastiaanssen, T.F., Peterson, V.L., Crispie, F., Cotter, P.D., Clarke, G., Cryan, J.F., O'Halloran, K.D., 2020. Prebiotic administration modulates gut microbiota and faecal short-chain fatty acid concentrations but does not prevent chronic intermittent hypoxia-induced apnoea and hypertension in adult rats. EBioMedicine 59, 102968.

O'Connor, K.M., Lucking, E.F., Golubeva, A.V., Strain, C.R., Fouhy, F., Cenit, M.C., Dhaliwal, P., Bastiaanssen, T.F., Burns, D.P., Stanton, C., 2019. Manipulation of gut microbiota blunts the ventilatory response to hypercapnia in adult rats. EBioMedicine.

Papalini, S., Michels, F., Kohn, N., Wegman, J., Van Hemert, S., Roelofs, K., Arias-Vasquez, A., Aarts, E., 2019. Stress matters: randomized controlled trial on the effect of probiotics on neurocognition. Neurobiology of stress 10, 100141.

Pearson-Leary, J., Zhao, C., Bittinger, K., Eacret, D., Luz, S., Vigderman, A.S., Dayanim, G., Bhatnagar, S., 2019. The gut microbiome regulates the increases in depressive-type behaviors and in inflammatory processes in the ventral hippocampus of stress vulnerable rats. Molecular psychiatry 1.

Provensi, G., Schmidt, S.D., Boehme, M., Bastiaanssen, T.F., Rani, B., Costa, A., Fouhy, F., Strain, C., Stanton, C., Blandina, P., 2019. Preventing adolescent stress-induced cognitive and microbiome changes by diet. Proceedings of the National Academy of Sciences, 201820832.

Ryan, F.J., Ahern, A., Fitzgerald, R., Laserna-Mendieta, E., Power, E., Clooney, A., O'Donoghue, K., McMurdie, P., Iwai, S., Crits-Christoph, A., 2020. Colonic microbiota is associated with inflammation and host epigenomic alterations in inflammatory bowel disease. Nature communications 11, 1–12.

Schwarcz, R., Bruno, J.P., Muchowski, P.J., Wu, H.-Q., 2012. Kynurenines in the mammalian brain: when physiology meets pathology. Nature Reviews Neuroscience 13, 465–477.

Silverman, J.D., Washburne, A.D., Mukherjee, S., David, L.A., 2017. A phylogenetic transform enhances analysis of compositional microbiota data. Elife 6, e21887.

Sommer, F., Bäckhed, F., 2013. The gut microbiota — masters of host development and physiology. Nat Rev Microbiol 11, 227.

Stilling, R.M., Moloney, G.M., Ryan, F.J., Hoban, A.E., Bastiaanssen, T.F., Shanahan, F., Clarke, G., Claesson, M.J., Dinan, T.G., Cryan, J.F., 2018. Social interaction-induced activation of RNA splicing in the amygdala of microbiome-deficient mice. eLife 7, e33070.

Stothart, M.R., Palme, R., Newman, A.E., 2019. It's what's on the inside that counts: stress physiology and the bacterial microbiome of a wild urban mammal. Proceedings of the Royal Society B 286, 20192111.

Strandwitz, P., Kim, K.H., Terekhova, D., Liu, J.K., Sharma, A., Levering, J., McDonald, D., Dietrich, D., Ramadhar, T.R., Lekbua, A., 2019. GABA-modulating bacteria of the human gut microbiota. Nature microbiology 4, 396–403.

Suez, J., Zmora, N., Zilberman-Schapira, G., Mor, U., Dori-Bachash, M., Bashiardes, S., Zur, M., Regev-Lehavi, D., Ben-Zeev Brik, R., Federici, S., Horn, M., Cohen, Y., Moor, A.E., Zeevi, D., Korem, T., Kotler, E., Harmelin, A., Itzkovitz, S., Maharshak, N., Shibolet, O., Pevsner-Fischer, M., Shapiro, H., Sharon, I., Halpern, Z., Segal, E., Elinav, E., 2018. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. Cell 174 (1406-1423), e1416.

Sugihara, G., May, R., Ye, H., Hsieh, C.-h., Deyle, E., Fogarty, M., Munch, S., 2012. Detecting causality in complex ecosystems. Science 338, 496–500.

Szyszkowicz, J.K., Wong, A., Anisman, H., Merali, Z., Audet, M.C., 2017. Implications of the gut microbiota in vulnerability to the social avoidance effects of chronic social defeat in male mice. Brain Behav Immun 66, 45–55.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., Raes, J., 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. Nature Microbiology.

van de Wouw, M., Boehme, M., Lyte, J.M., Wiley, N., Strain, C., O'Sullivan, O., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2018. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain–gut axis alterations. J Physiol 596, 4923–4944.

Walter, J., Armet, A.M., Finlay, B.B., Shanahan, F., 2020. Establishing or exaggerating causality for the gut microbiome: lessons from human microbiota-associated rodents. Cell 180, 221–232.

Wang, S., Qu, Y., Chang, L., Pu, Y., Zhang, K., Hashimoto, K., 2020. Antibiotic-induced microbiome depletion is associated with resilience in mice after chronic social defeat stress. Journal of affective disorders 260, 448–457.
Weinstock, G.M., 2011. The volatile microbiome. Genome Biol 12, 114-114.
Xu, M., Wang, C., Krolick, K.N., Shi, H., Zhu, J., 2020. Difference in post-stress recovery of the gut microbiome and its altered metabolism after chronic adolescent stress in rats. Scientific Reports 10, 1–10.
Zijlmans, M.A., Korpela, K., Riksen-Walraven, J.M., de Vos, W.M., de Weerth, C., 2015. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology 53, 233–245.