Gut microbial diversity and inferred capacity to produce butyrate modulate cortisol reactivity following acute stress in healthy adults

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Abstract

Acute stress triggers the release of stress hormones such as cortisol, increasing stress reactivity and aiding post-stress recovery. Prior work in rodents revealed the modulating role of the gut microbiota in stress reactivity, but whether this is also the case in humans is unclear. Additionally, to what degree stress reactivity is tied to one's capacity to produce microbial metabolites such as short-chain fatty acids (SCFAs) is untested. To close this gap, we invited 80 healthy human adults to the laboratory who were either exposed to a well-established, standardized intervention that induced acute stress or to a non-stressful control condition (n = 40 per group). Changes in stress hormones were assessed from repeated saliva sampling. Stool samples were obtained at baseline, and the gut microbiota were characterized through 16S rRNA gene amplicon sequencing. We found that higher gut microbiota diversity was associated with lower cortisol stress reactivity and lower levels of subjectively experienced stress, but not faster post-stress recovery, across the individuals of the stress group. Moreover, lower cortisol stress reactivity was associated with a higher relative abundance of taxa that encode metabolic pathways for the production of butyrate, a key SCFA. These results are the first to highlight the role of gut microbial diversity and inferred butyrate production capacity in modulating stress reactivity in healthy adults, underscoring the microbiota's potential to buffer against the detrimental effects of acute stress.

Keywords

Acute stress, cortisol, human, gut microbiota, short-chain fatty acids (SCFAs), butyrate

1. Introduction

Acute stress describes the allostatic processes that cause a deviation from bodily homeostasis in response to a perceived threat or challenge (McEwen, 2017; Sapolsky, 2019). On a physiological level, stress is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, which titrates the release of stress hormones such as cortisol (De Kloet et al., 2005). Encountering an acute stressor initiates a rise in cortisol (typically peaking after approx. 20 minutes; Dickerson & Kemeny, 2004; Linz et al., 2019), after which it returns to baseline (approx. 90 minutes post-stressor; Kirschbaum & Hellhammer, 1994; Linz et al., 2019). Adequate and time-limited cortisol stress reactivity and post-stress recovery are important markers of an adaptive stress response and are altered in anxiety-related disorders and depression (Fiksdal et al., 2019; Zorn et al., 2017). Emerging evidence suggests that the microorganisms that inhabit the gastrointestinal tract – collectively termed the gut microbiota – play a key role in modulating the stress response (Cryan et al., 2019; Foster et al., 2017). For instance, Sudo and colleagues (2004) found that germ-free mice, which lack a gut microbiota, showed exaggerated stress reactivity that could be normalized through colonization with the bacterium *Bifidobacterium infantis,* a probiotic bacterium known to confer health benefits to the host. Gut microbiota composition can be characterized through diversity (which accounts for the number and/or relative abundance of genetically distinct bacterial taxa). While the overall gut microbiota composition appears highly specific to an individual (Yatsunenko et al., 2012), patients with major depressive disorder were shown to exhibit altered gut microbiota composition (e.g., decreased bacterial diversity compared to healthy controls). Moreover, transplantation of their fecal material to microbiota-depleted rats induced depression-like behavior in these animals (Kelly et al., 2016). In humans, probiotic (thus, beneficial) gut bacteria were shown to buffer against the detrimental effects of acute stress on cognition (Papalini et al., 2019) and negative emotions (Steenbergen et al., 2015). Whether gut microbiota composition is linked to cortisol stress reactivity and post-stress recovery in healthy adults is, however, unclear.

One pathway of how the gut microbiota can affect stress-related neurophysiology is through the production of metabolites such as short-chain fatty acids (SCFAs; Dalile et al., 2019, 2020; O'Riordan et al., 2022). SCFAs are mainly derived from the fermentation of dietary (Casterline et al., 1997; Slavin, 2013) and prebiotic fibers (Davani-Davari et al., 2019; Rastall & Gibson, 2015) are produced by health-associated and probiotic bacteria (Grimaldi et al., 2017), and are central to maintaining gut health, metabolism, and immune function (Wenzel et al., 2020). Supplementation with a mixture of the key SCFAs acetate, propionate, and butyrate (Van De Wouw et al., 2018), or only with butyrate (Wang et al., 2023) was shown to decrease corticosterone release following acute stress induction in chronically stressed mice and in an autism rat model, respectively. In humans, Dalile and colleagues (2020) found that supplementation with the abovementioned SCFA mixture altered SCFA serum levels and attenuated cortisol release after psychosocial stress induction (Dalile et al., 2020). Moreover, the administration of prebiotics, which are known to increase SCFA-producing bacteria as well as SCFA concentrations (Davani-Davari et al., 2019; Grimaldi et al., 2017), was shown to reduce the cortisol awakening response and partly decreased attentional vigilance to negative information (Schmidt et al., 2015). Nevertheless, a clear understanding of whether and to what degree an individual's gut microbial capacity to produce SCFAs modulates cortisol stress reactivity and post-stress recovery is missing. Addressing this gap could help identify microbial targets to enhance stress resilience, potentially informing dietary or microbiome-based interventions.

In the present study, we invited 80 healthy human participants to the laboratory. Each donated a stool sample for characterization of the gut microbiota in terms of overall composition (using 16S rRNA gene amplicon sequencing) and inferred capacity to produce SCFAs. Participants were then randomly assigned to a stress or control group and underwent an acute stress induction procedure or a non-stressful control intervention, respectively (using a modified version of the Montreal Imaging Stress Task, MIST; Dedovic et al., 2005). Cortisol stress reactivity and post-stress recovery were quantified by repeated salivary cortisol sampling, paralleled by assessments of participants' subjective stress experience (Figure 1A). We hypothesized that cortisol stress reactivity and post-stress recovery would be linked to gut microbiota composition across participants. Specifically, we expected that higher gut microbiota diversity would be associated with lower cortisol stress reactivity and faster post-stress recovery. Because the gut microbiota was shown to impact stress-related neurophysiology via metabolite production of SCFAs (Van De Wouw et al., 2018), we expected that higher gut microbial capacity to produce SCFAs would be coupled with lower cortisol stress reactivity and faster post-stress recovery across participants. Lastly, since both the acute stress response (Adjei et al., 2018; Stefanaki et al., 2022) and gut microbiota composition (Stefanaki et al., 2022) depend on biological sex, we explored whether the obtained results differed between biological males and females.

2. Methods

2.1. Participants

Eighty healthy participants were recruited through the University of Vienna's online recruitment platform and were invited to the laboratory (62.5% females, age range: 18-34 years). Participants had no history of neurological or psychiatric diagnosis and did not report any gastrointestinal diseases or symptoms thereof (such as prolonged diarrhea or constipation within the last 3 months). The use of antibiotics, probiotics, or other medications (e.g., steroids, cytokines, proton pump inhibitors) within the past three months was an exclusion criterion since these substances were shown to influence the gut microbiota (Palleja et al., 2018; Vich Vila et al., 2020; Zmora et al., 2018). Also, the intake of hormonal contraceptives was an exclusion criterion, since they were shown to blunt stress hormone levels in females (Kirschbaum et al., 1999; Nielsen et al., 2013). The majority of participants were University students who received course credits or monetary compensation. Four participants were excluded from the analyses because they did not understand the experimental instructions and/or did not engage with the task, and one participant was excluded due to technical difficulties. This resulted in a final sample size of 75 participants who were randomly assigned to the stress group (n=36 participants, 72% females, age range: 18-34 years, mean age: 22 years) or the control group (n=39, 49% females, age range: 18-33 years, mean age: 23 years). All participants provided written informed consent prior to entering the study. The study procedures were conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013) and were approved by the ethics committee of the University of Vienna, Austria.

2.2. Study design

The study took place across two consecutive study days (Figure 1A). For measurements of salivary cortisol, participants were instructed to refrain from food and caffeine intake (they were asked to only drink water, but no caffeinated drinks), from brushing their teeth, and from chewing gum up to 2 hours before the start of the experimental sessions on both days. They were also instructed to refrain from physical activity (sports), cigarette smoking, alcohol

consumption, and consumption of drugs or medications for up to 24 hours before the start of the first experimental session on day 1 until the end of their study participation on day 2.

On day 1, participants completed initial ratings (T_0) of their subjective stress experience and their mood ("In this moment I am feeling…?", items: "stress", "happy", "bad", "anxious", "disgust", "sad", "calm", "surprise", "angry", response via a 7-point Likert scale ranging from "not at all" to "very"). They provided a saliva sample (~20 minutes after arrival at the laboratory) and were instructed on how to perform the stool sampling (see below for details on the sampling procedures).

On day 2, participants received the task instructions depending on whether they belonged to the stress or control group. Since this study was part of a larger project that tested the effects of acute stress on the neural underpinnings of spatial navigation, participants then completed a (non-stressful) object-location task (Doeller et al., 2010) within the MR scanner that consisted of four separate task runs (~15 minutes each, not further discussed here). The acute stress induction took place ~30 minutes after the start of the MRI measurements (i.e., after 2 task runs of the object-location task) inside the MR scanner (see below for details). After ~45 minutes (i.e., after the third task run of the object-location task), another acute stress induction took place that acted as a "stress reminder". This was done to ensure that both remaining runs of the object-location task would be impacted equally by the acute stress induction (Forbes et al., 2024; Vogel et al., 2015). Overall, participants' subjective stress experience and salivary cortisol were assessed at six time points on day 2 (approx. minutes pre/post main stress induction; T_1: -57 min; T_2: 0 min (stress induction); T_3, +23 min; T_4, +40 min; T_5, +59 min; T_6, +84 min). To account for inter-individual differences in trait anxiety and perceived stress, participants further completed online versions of the General Anxiety Disorder-7 (GAD-7; Williams, 2014) and the Perceived Stress Scale (PSS; Cohen, 1988).

2.3. Acute stress induction

On day 2, participants of the stress group were exposed to a modified version of the Montreal Imaging Stress Task (MIST) that combined an arithmetic challenge with social evaluation (Dedovic et al., 2005; Forbes et al., 2024). At the beginning of the session, participants were told the cover story, which was that the study revolved around the link between arithmetic performance and cognition and that it was important that they performed well on the arithmetic challenge. They were told that their performance would be video recorded and evaluated. The participants then completed two task runs of the unrelated object-location task, after which the main acute stress intervention took place. This comprised two runs of the computerized arithmetic challenge (6 minutes each), during which participants solved arithmetic problems of increasing difficulty under time pressure. They were shown a live stream via webcam of the experimenter taking notes. Following a standardized feedback protocol, the experimenter gave negative feedback on participants' performance and told them that their data was unusable if their performance was too low compared to the alleged group average. At certain times, the experimenter interrupted the arithmetic challenge and told participants that the task needed to be started again due to their insufficient performance. The experimenters were wearing white lab coats throughout the session.

After the arithmetic challenge, participants completed a counting task (3 minutes) that was adapted from the Trier Social Stress Test (TSST; Kirschbaum et al., 1993). The experimenter entered the MRI scanner room, stood next to the scanner bed, and instructed the participant to count down from 2043 in steps of 17. They were instructed to start over when making a mistake and to respond faster, maximizing pressure through social evaluation. Experimenters also attempted to distract them from counting by urging them to respond faster or speak more loudly while they were calculating, increasing the likelihood of errors. The experimental session

commenced with the third run of the object-location task and another (shorter) acute stress intervention that only included the arithmetic counting task, but with different numbers.

The control group participants were told the same cover story but underwent a non-stressful version of the MIST. They were not told that their performance would be video recorded and evaluated. While the control group still completed the arithmetic challenge with matched difficulty and overall duration, no video live stream of the experimenter was shown, and no negative feedback was provided. The experimenters also did not wear white lab coats during the session. The instructions for the counting task were given via the MRI scanner intercom rather than in person, and participants were counting quietly in their heads from 0 in steps of 5, and, after 3 minutes, reported the number they had reached. The counting task was repeated after the third run of the object-location task. After the study completion, all deception was resolved, and participants were told about the actual study goal.

2.4. Salivary cortisol sampling and analysis

For salivary cortisol sampling, participants were instructed to keep the cotton pad in their mouth for 60 seconds, without chewing (Salivette, Sarstedt, Germany). The collected samples were refrigerated for the duration of the experimental session and were subsequently transferred to freezer storage (-25 °C). Upon completion of data collection for the entire study, samples were shipped to the laboratory of Prof. Kirschbaum (Technical University Dresden, Germany) and were analyzed using a commercially available chemiluminescence immunoassay with high sensitivity (IBL International, Hamburg, Germany). Of the 560 samples collected, 537 were analyzed in duplicates, 16 were analyzed as singlets due to limited saliva volume, and 7 were excluded due to insufficient saliva volume. Based on the duplicates, we calculated the mean cortisol concentration and log-transformed the values for all following analyses.

To obtain participants' cortisol stress reactivity in response to the main part of the acute stress intervention, we calculated the difference between the cortisol value that was sampled directly prior to the acute stressor (at T_2), which served as the pre-stress baseline, and the peak cortisol value post-stress (either at T_3 or T_4). To account for interindividual variability in the time interval between sampling time points (because the exact duration of the object-location task runs was dependent on participants' performance, leading to slight timing differences between individuals), the resulting differences were normalized to the time interval between sample collections. This peak-minus-baseline approach is consistent with established procedures in stress research (e.g., Miller et al., 2013; Schlotz et al., 2008).

$$Cortisol\ Stress\ Reactivity = \frac{\max(T_3/T_4) - T_2}{Time\ in\ minutes(\max(T_3/T_4) - T_2)}$$

To determine how quickly participants' post-stress cortisol decreased to their pre-stress cortisol levels (i.e., their stress recovery parameter), we calculated the difference between the abovementioned peak value (either at T_3 or T_4) and the last sampling timepoint following the acute stress phase on day 2 (T_5), again, normalized to the time interval between sample collections:

$$Cortisol\ Stress\ Recovery = \frac{T_5 - \max(T_3/T_4)}{Time\ in\ minutes(T_5 - \max(T_3/T_4))}$$

2.5. Subjective stress analysis

To quantify participants' subjective stress reactivity in response to the acute stressor, we calculated the difference between the subjective stress rating obtained immediately before the acute stress phase (T_2) and the peak subjective stress rating obtained after the stressor (either

at T_3 or T_4). As above, to account for interindividual differences in the time interval between sampling time points, the difference was normalized by the time interval between sample collections:

$$Subjective\ Stress\ Reactivity = \frac{\max(T_3/T_4) - T_2}{Time\ in\ minutes(\max(T_3/T_4) - T_2)}$$

2.6. Stool sampling and analysis

Stool samples were self-sampled by participants using a home-collection kit (Sarstedt, Germany) that was provided to them on day 1 (including gloves, the sampling tube with a spatula, a gel cooling pad, and a Styrofoam container for transport). Participants were asked to freeze the sample and transport it back to the laboratory on study day 2, using the pre-cooled gel pad and Styrofoam container to prevent thawing. Samples were frozen (-25 °C) and, after all data collection was completed, were shipped to the Joint Microbiome Facility (University of Vienna and Medical University of Vienna, Austria) for 16S rRNA gene amplicon sequencing according to an established protocol (Pjevac et al., 2021). Along with sample collection, participants completed several questionnaires regarding their stool quality (Bristol Stool Chart; Lewis & and Heaton, 1997), dietary habits, and other lifestyle factors that can influence the gut microbiota (Johnson et al., 2020; Quigley, 2017), including screening questions that were partly based on the Human Gut Microbiome Project (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/document.cgi?study_id=phs000228.v3.p1&phd=2844).

DNA from the stool samples was extracted using the QIAamp DNA Stool Mini Kit automated on a QiaCube (Qiagen, The Netherlands). For microbial community profiling, the 16S rRNA genes were amplified by polymerase chain reaction (PCR) using primers that cover most of the bacterial and archaeal clades (515F/806R; (Apprill et al., 2015). Amplicons were barcoded, multiplexed, and sequenced on an Illumina MiSeq platform (Illumina, United States; Herbold et al., 2015). Negative controls were included to monitor potential contamination. Raw sequencing data were quality-filtered, demultiplexed, and processed using DADA2 (Callahan et al., 2016) for obtaining amplicon sequence variants (ASVs). ASVs were taxonomically classified using SINA (Pruesse et al., 2012) using the SILVA SSU rRNA database (Version 138.1; Quast et al., 2013). If necessary, contaminants were removed *in silico* using the decontam software package (Davis et al., 2018).

To account for differences in sequencing depth and ensure comparability between individuals, we inspected the distribution of sequencing depths across all samples and rarefied the data to the minimum library size (5991). ASVs with a relative abundance < 0.1% were excluded from the samples to minimize the contribution of extremely rare sequences that may reflect sequencing noise. To determine participants', gut microbial community composition, ASV abundance data were analysed in terms of different alpha diversity metrics, such as Shannon, Inverse Simpson, and the number of observed ASVs (i.e., richness).

To quantify each individual's inferred capacity to produce SCFAs, we identified SCFA-producing bacterial taxa based on the taxonomic classification of SCFA-producing bacteria provided by Frolova et al. (2022). Their classification was derived from a metagenomic reconstruction of SCFA biosynthesis pathways across 2,856 reference genomes (see supplement, Table S1). Taxa were included in our analysis only if they were reported to produce butyrate or propionate (we did not analyze acetate because this biosynthesis pathway is highly abundant and is present in almost all gut bacteria; Frolova et al., 2022; Louis & Flint, 2017). Some bacterial taxa possess multiple SCFA biosynthesis pathways for the same SCFA

– these were counted once, treating SCFA production as a binary phenotype rather than a quantitative measure of pathway abundance.

2.7. Statistical analysis

All analyses were conducted in R (version 4.4.0 R Core Team, 2024) in combination with "tidyverse" (version 2.0.0; Wickham et al., 2019) and Bioconductor (version 3.19; Huber et al., 2015). Microbiome data analysis was conducted using the "ampvis2" (version 2.8.3; Andersen et al., 2018) and "phyloseq" (version 1.48.0; McMurdie & Holmes, 2013) packages. Statistical tests and multiple comparison corrections were performed using "rstatix" (version 0.7.2; Alboukadel Kassambara, 2021). Separate independent-samples *t*-tests and analyses of variance (ANOVAs) were used to compare the groups and to analyze group × time interactions of salivary cortisol and subjective stress levels, respectively. Effect sizes for *t*-tests were calculated as Cohen's *d* (Lakens, 2013). Unless stated otherwise, the α-level was set to 0.05 (two-tailed) for all analyses; results with $p < 0.1$ were considered as a statistical trend. Analyses were corrected for multiple comparisons when appropriate, using the Bonferroni-Holm correction. Results were visualized using "ggplot2" (version 3.5.1; Hadley, 2016), and model effects were illustrated using the "effects" package (version 4.2-2; Fox & Weisberg, 2018, 2019). Statistical tables were generated using the "stargazer" package (version 5.2.3; Hlavac, 2022).

2.7.1. Model definition, evaluation, and comparison

To determine the effect of relevant predictors and covariates on cortisol stress reactivity, post-stress recovery, and subjective stress levels, we initially defined linear models (LMs) using the "stats" package (version 4.4.0; R Core Team, 2024b). However, due to violations of normality, heteroscedasticity, and to alleviate the effect of potential outliers, we subsequently used robust linear models (RLMs) with Huber's loss function using the "MASS" package (version 7.3-61; Venables & Ripley, 2002). We computed a corresponding LM for each RLM to validate model stability and compared the explained variance (variance-based R^2 measure for RLMs), residual distributions, and sensitivity to outliers between models.

Model coefficients were tested for significance with the "coeftest" function from the "lmtest" package (version 0.9-40; Zeileis & Hothorn, 2002). To evaluate model fit, we calculated a variance-based R^2 measure for RLMs (using the "rlm" function from the "MASS" package), analogous to the coefficient of determination (R^2) for ordinary least squares regression. The variance-based R^2 measure quantifies the proportion of variance explained by the model while down-weighting and accounting for the influence of potential outliers, computed as variance-based $R^2 = 1 - SS_{res} / SS_{tot}$ (where SS_{res} denotes the sum of squared residuals, and SS_{tot} denotes the total sum of squares, based on the mean value of the dependent variable). To ensure model robustness, we conducted outlier diagnostics using Cook's distance (with the standard threshold of 4/n) and inspected the residuals. This confirmed that extreme values did not disproportionately influence the model estimates, arguing for the stability and reliability of the selected RLMs. Finally, we compared the RLMs to their corresponding LMs regarding their explained variance (variance-based R^2 measure for RLMs), residual distributions, and sensitivity to outliers. Across all models, RLMs demonstrated higher explained variance, residuals were distributed symmetrically, and skewness was reduced compared to standard LMs.

2.7.2. Selection of maximal and base models

Model selection followed a stepwise approach inspired by established guidelines for confirmatory modeling (Barr et al., 2013), beginning with a maximal model including all theoretically relevant predictors. Although these recommendations were originally developed

for mixed-effects models, we applied the same principled approach to robust linear models, simplifying them iteratively to achieve convergence and interpretability. Specifically, we began with a maximal model that predicted the three dependent variables (cortisol stress reactivity, post-stress recovery, subjective stress reactivity) using all relevant variables, including: biological sex, perceived stress (PSS), trait anxiety (GAD-7) and baseline cortisol (T_2) for cortisol stress reactivity and post-stress recovery.

Maximal model:

Dependent variable ~ biological sex+ general anxiety+ perceived stress + subjective_stress+ baseline cortisol (T_2; the latter was only included for cortisol stress reactivity and post-stress recovery)

From the maximal model, non-significant variables were removed iteratively until the model converged and the maximum explained variance was achieved. This resulted in the base model that predicted the three dependent variables (cortisol stress reactivity, post-stress recovery, subjective stress reactivity).

Base model:

Dependent variable ~ biological sex + general anxiety + perceived stress + baseline cortisol (T_2) (the latter was only included for cortisol stress reactivity and post-stress recovery)

Results pertaining to the base model revealed a significant effect of biological sex on cortisol stress reactivity, with biological males showing generally lower cortisol stress reactivity than biological females (b=-0.040, SE = 0.018, z = -2.27 p = 0.023). The model explained approximately 10.3% of the variance in cortisol reactivity (variance-based R^2 = 0.103). Turning to the post-stress recovery of salivary cortisol, biological males exhibited significantly steeper cortisol recovery slopes than biological females (b = -0.015, SE = 0.007, z = - 2.16, p=0.031). Moreover, post-stress recovery of salivary cortisol was significantly faster at higher levels of general anxiety (b = -0.013, SE = 0.005, z = 2.59, p = .010) and higher baseline cortisol (b=-0.020, SE = 0.003, z = -6.27, p < 0.001). The predictors explained approximately 25.7% of the variance in post-stress recovery slopes (variance-based R^2 = 0.257). Finally, we examined predictors of subjective stress reactivity. In the base model, general anxiety emerged as a significant predictor, with higher trait anxiety being associated with increased subjective stress responses (b = 0.043, SE = 0.019, z = 2.223, p = .026). Neither biological sex (p = .153) nor perceived stress (p = .132) significantly predicted subjective stress reactivity. The model explained approximately 6.5% of the variance in subjective stress reactivity (variance-based R^2 = 0.065).

Together, the base models allowed us to establish a reference point for evaluating the added explanatory value of gut microbiota parameters and microbiota-related variables in the following steps.

2.7.3. Selection of gut microbiota models

Lastly, we defined separate RLMs that incorporated the gut microbial predictors and additional microbiota-related variables into the final base model. Similar to the approach described in section 2.7.2, we once again started from a maximal model that included gut microbiota predictors. Non-significant variables were iteratively removed until the model converged, and the maximum explained variance was achieved. This approach allowed us to examine whether variance in the dependent variables covariied with individual differences in gut microbiota composition across individuals. The gut microbial predictors included alpha diversity (either Shannon or Inverse Simpson indices, or the observed number of ASVs), and the microbiota-related variables such as diet, body-mass index (BMI), birth mode (vaginal or caesarean

section) and early nutrition (breastfed, bottle fed, or both). We also defined a separate RLM that included the individual Firmicutes/Bacteroidetes (F/B) ratio as a covariate, as this has been linked to stress-related physiological processes and gut microbiota composition in previous studies (Jiang et al., 2015; Lach et al., 2018; Pusceddu et al., 2015). Finally, we used the same approach to assess how the relative abundance of SCFA-producing taxa covariied with the variance in the dependent variables across individuals.

Gut microbiota model:

Dependent variable ~ gut microbiota measure (e.g., alpha diversity, F/B ratio, SCFA-producing taxa) + biological sex + general anxiety + perceived stress + baseline cortisol (T_2) (the latter was only included for cortisol stress reactivity and post-stress recovery)

3. Results

3.1. Acute stress intervention effectively increases salivary cortisol and subjective stress

Starting out, we verified that the stress and control groups were comparable in terms of age, biological sex, BMI, perceived stress (PSS; mean±standard error of the mean, s.e.m., stress group: 18.305±1.147, controls: 16.128±1.108), and trait anxiety (GAD-7; stress group: 6.361±0.668, controls: 5.717±0.709; all $p > 0.05$). To verify that the acute stress induction affected stress-related measures, we conducted a repeated-measures ANOVA on the log-transformed salivary cortisol values with group (stress vs. control) as a between- and timepoint (7 sampling timepoints across both days) as a within-subjects factor. The analysis revealed a significant group × timepoint interaction ($F(3.29, 240.11) = 3.629$, $p = 0.011$, $\eta^2 = 0.011$; main effect of group: $F(1.00, 73.00) = 5.473$, $p = 0.022$, $\eta^2 = 0.055$; main effect of timepoint: $F(3.29, 240.11) = 12.011$, $p < 0.001$, $\eta^2 = 0.036$). As expected, post-hoc *t*-tests showed comparable cortisol levels between the groups before acute stress induction (T_1-T_2; all $p > 0.05$), and significantly higher cortisol levels after stress in the stress group compared to controls (T_3-T_6; all $p < 0.05$, corrected for multiple comparisons, Fig. 1B; Table 1).

Similarly, subjective stress levels showed a significant group × timepoint interaction ($F(6, 420) = 2.440$, $p = 0.025$, $\eta^2 = 0.025$), a significant main effect of timepoint ($F(6, 420) = 3.025$, $p = 0.007$, $\eta^2 = 0.031$), but no significant main effect of group ($F(1, 70) = 1.661$, $p = 0.202$, $\eta^2 = 0.006$). Post-hoc *t*-tests revealed comparable stress levels between the groups before acute stress induction (T_1–T_2; all $p > 0.05$) and significantly increased subjective stress in the stress group compared to the controls after stress induction (T3, $p = 0.012$, corrected for multiple comparisons), which recovered relatively quickly (T_4-T_6; all $p > 0.0.05$, Fig. 1C; Table 1). Together, these results highlight that the acute stress intervention successfully increased salivary cortisol and subjective stress levels in the stress group compared to the control group.

Table 1. Main effect of group on cortisol and subjective stress per timepoint

One-way ANOVAs testing the effect of group (stress vs. control) on cortisol levels and subjective stress ratings at each sampling timepoint (see also Figure 1A). Only post-stress timepoints (T3-T6) were adjusted for multiple comparisons (using Bonferroni-Holm correction). P < .05 (adjusted) is considered statistically significant (*).

Timepoint	Label	Effect	DFn	DFd	F	*p*	ges	p.adj
Main effect of group on cortisol								
0	Day 1	group	1	73	0.402	0.528	0.005	0.528
1	Day 2 – T1	group	1	73	1.703	0.196	0.023	0.456
2	T2	group	1	73	2.093	0.152	0.028	0.456
3	T3	group	1	73	5.218	0.025	0.067	0.048*
4	T4	group	1	73	10.268	0.002	0.123	0.006*
5	T5	group	1	73	11.191	0.001	0.133	0.004*
6	T6	group	1	73	5.353	0.024	0.068	0.048*
Main effect of group on subjective stress ratings								
0	Day 1	group	1	73	0.216	0.643	0.003	1
1	Day 2 – T1	group	1	73	0.819	0.369	0.011	1
2	T2	group	1	73	0.752	0.389	0.01	1
3	T3	group	1	73	9.63	0.003	0.117	0.012*
4	T4	group	1	73	0.63	0.43	0.009	0.966
5	T5	group	1	71	0.995	0.322	0.014	0.966
6	T6	group	1	72	0.021	0.886	0.0003	0.966

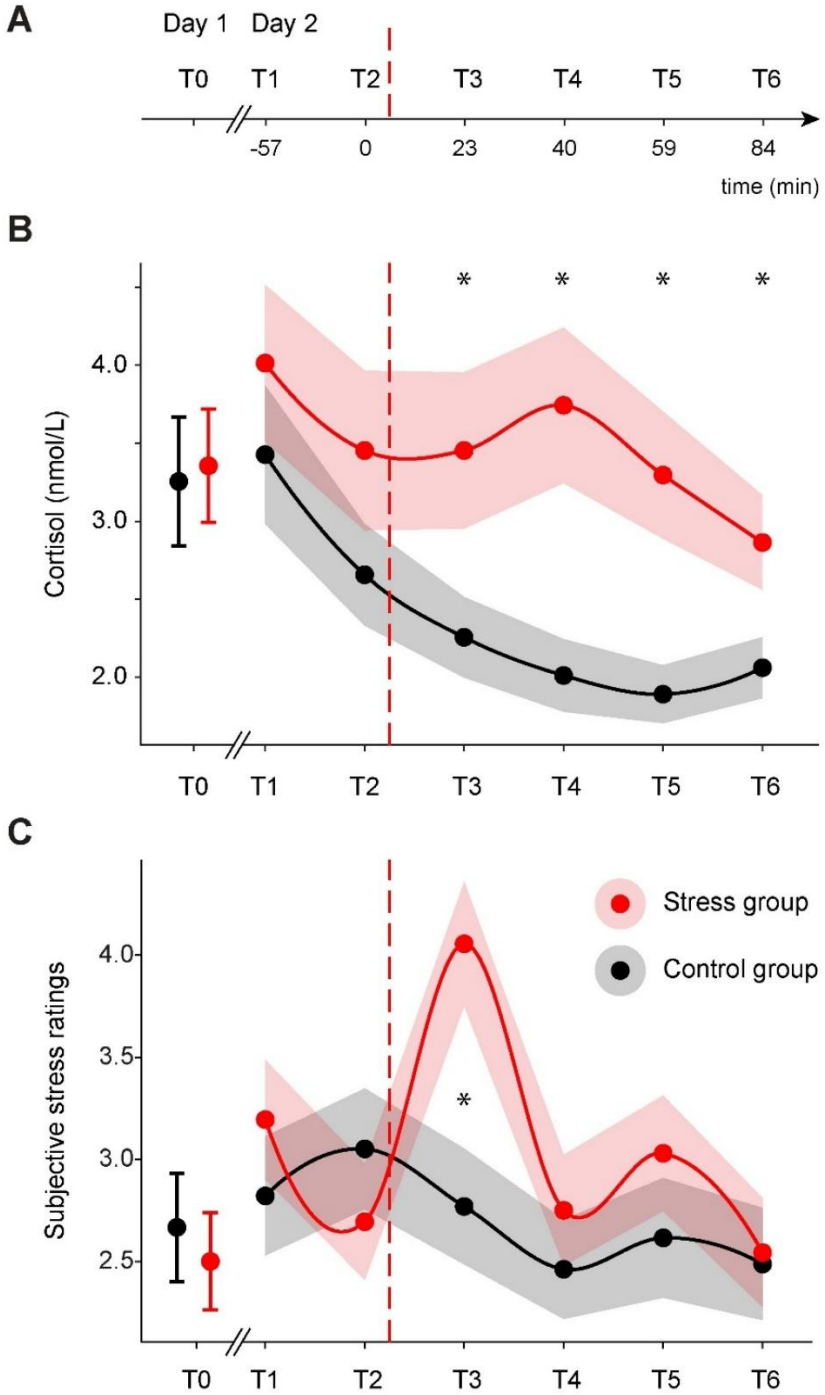


Figure 1. Study design, salivary cortisol, and subjective stress ratings. (A) Study design and timing of the acute stress intervention (red dashed line; see Methods section for details). Time is displayed in minutes relative to the onset of the acute stress intervention (T2 = 0 min). Note that a stress reminder was given after T4 (not shown, see Methods section for details). **(B)** Salivary cortisol concentrations (nmol/L, note that statistical comparisons were performed using the log-transformed cortisol values), and **(C)** subjective stress ratings of the stress (red) and control group (black). Continuous lines depict the respective group average, and shaded areas represent the standard error of the mean (SEM). * denotes significant group differences ($p < .05$).

3.2. Gut microbial alpha diversity is associated with cortisol stress reactivity

Our main goal was to determine whether inter-individual differences in gut microbiota composition explained variation in participants' responses to acute stress (Cryan et al., 2019; Foster et al., 2017). Specifically, we hypothesized that participants with higher gut microbial diversity would exhibit lower cortisol reactivity and a faster post-stress recovery. To test this, we quantified cortisol stress reactivity as the slope of cortisol increase following the acute stress intervention for all individuals of the stress group (see Methods section for details). We next examined whether different metrics of gut microbial alpha diversity (i.e., Shannon, Inverse Simpson indices, and the observed number of ASVs) were associated with cortisol stress reactivity (note that there were no significant differences in any of the alpha diversity metrics between the groups, all $p > 0.05$).

Results showed that all three alpha diversity metrics significantly predicted cortisol stress reactivity (separate RLMs, $N = 36$ each). Specifically, higher Shannon diversity was associated with lower stress reactivity across participants ($b = -0.016$, $SE = 0.057$, $z = -2.916$, $p = .003$; variance-based $R^2 = 0.151$). Similarly, higher Inverse Simpson diversity ($b = -0.017$, $SE = 0.005$, $z = -3.2107$, $p = .001$; variance-based $R^2 = 0.148$) and a higher number of observed ASVs (thus, higher gut microbial richness) showed a significantly negative association with cortisol stress reactivity across participants ($b = -0.016$, $SE = 0.008$, $z = -2.097$, $p = .03$; variance-based $R^2 = 0.186$). Biological males showed generally lower cortisol stress reactivity across all models (all $p < 0.001$; Figure 2A-C, see Table 2).

Next, our rationale was that gut microbiota diversity may not only buffer against stress reactivity but also support faster post-stress recovery, which reflects how efficiently the HPA axis returns the body to homeostasis after a stressor (McEwen, 1993; Sapolsky et al., 2000; Sudo et al., 2004). To capture this, we quantified post-stress recovery as the slope of cortisol decline after the stress peak (see Methods section for details). We then repeated the abovementioned analysis and examined the relationship between post-stress recovery and the different alpha diversity metrics.

In contrast to cortisol stress reactivity, none of the alpha diversity metrics were significantly associated with post-stress stress recovery (Shannon diversity and observed number of ASVs: variance-based $R^2 = 0.265$, Inverse Simpson: variance-based $R^2 = 0.261$). However, a higher number of observed ASVs (thus, higher richness) was trend-level associated with faster post-stress recovery of salivary cortisol ($b = -0.006$, $SE = 0.003$, $z = -1.897$, $p = .0577$). Apart from gut microbial alpha diversity, higher baseline cortisol levels (all models $p < .001$) and male sex (all models $p < .05$) were significantly associated with faster post-stress recovery across individuals. Additional psychological variables also showed significant associations with post-stress recovery. Higher trait anxiety was linked to faster post-stress recovery (all $p < .001$), while higher levels of perceived stress were associated with slower post-stress recovery only in some models (Figure 2D, see Table 3 for an overview).

Taken together, results indicated that higher gut microbial alpha diversity was significantly associated with lower cortisol stress reactivity across participants. While there were no significant associations between gut microbial alpha diversity and post-stress recovery of salivary cortisol, we detected a clear trend toward higher gut microbial richness being tied to faster post-stress cortisol recovery across participants.

Table 2. Robust linear models (Microbiome diversity) predicting cortisol stress reactivity

Results from robust linear regression models predicting cortisol stress reactivity based on microbiome diversity (Shannon Index/Inverse Simpson/Observed), baseline cortisol, sex, and psychological variables (general anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$. Model formulas: Cortisol stress reactivity ~ biological sex + general anxiety + perceived stress + Shannon/Inverse Simpson/Observed + baseline cortisol (T_2).

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (Shannon) predicting cortisol stress reactivity					
(Intercept)	0.002	0.006	0.267	0.79	
Biological sex	-0.044	0.012	-3.774	0.0002	***
General anxiety	-0.009	0.008	-1.071	0.284	
Perceived stress	0.009	0.008	1.146	0.252	
Shannon	-0.016	0.006	-2.916	0.004	**
baseline cortisol (T_2)	-0.002	0.005	-0.473	0.636	
RLM (Inverse Simpson) predicting cortisol stress reactivity					
(Intercept)	0.0002	0.006	0.027	0.978	
Biological sex	-0.037	0.011	-3.430	0.001	***
General anxiety	-0.011	0.008	-1.456	0.145	
Perceived stress	0.011	0.008	1.492	0.136	
Inverse Simpson	-0.017	0.005	-3.211	0.001	**
baseline cortisol (T_2)	-0.004	0.005	-0.877	0.380	
RLM (Observed predicting cortisol stress reactivity					
(Intercept)	0.001	0.008	0.119	0.906	
Biological sex	-0.042	0.016	-2.681	0.007	**
General anxiety	-0.015	0.011	-1.327	0.185	
Perceived stress	0.013	0.011	1.153	0.249	
Observed	-0.017	0.008	-2.098	0.036	*
baseline cortisol (T_2)	0.001	0.007	0.107	0.915	

Table 3. Robust linear models (Microbiome diversity) predicting post-stress recovery

Results from robust linear regression models predicting post-stress recovery based on microbiome diversity (Shannon Index/Inverse Simpson/Observed), baseline cortisol, sex, and psychological variables (general anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$. Model formulas: Post-stress recovery ~ biological sex + general anxiety + perceived stress + Shannon/Inverse Simpson/Observed + baseline cortisol (T_2).

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (Shannon) predicting post-stress recovery					
(Intercept)	-0.016	0.004	-4.238	0.00002	***
Biological sex	-0.018	0.007	-2.433	0.015	*
General anxiety	-0.014	0.005	-2.726	0.006	**
Perceived Stress	0.009	0.005	1.715	0.086	
Shannon	-0.004	0.003	-1.237	0.216	
baseline cortisol (T_2)	-0.020	0.003	-6.344	0	***
RLM (Inverse Simpson) predicting post-stress recovery					
(Intercept)	-0.016	0.004	-4.234	0.00002	***
Biological sex	-0.016	0.007	-2.212	0.027	*
General anxiety	-0.014	0.005	-2.720	0.007	**
Perceived Stress	0.009	0.005	1.723	0.085	
Inverse Simpson	-0.004	0.004	-0.989	0.323	
baseline cortisol (T_2)	-0.021	0.003	-6.125	0	***
RLM (Observed) predicting post-stress recovery					
(Intercept)	-0.015	0.003	-4.473	0.00001	***
Biological sex	-0.017	0.007	-2.571	0.010	*
General anxiety	-0.016	0.005	-3.397	0.001	***
Perceived Stress	0.011	0.005	2.266	0.023	*
Observed	-0.006	0.003	-1.898	0.058	
baseline cortisol (T_2)	-0.020	0.003	-6.877	0	***

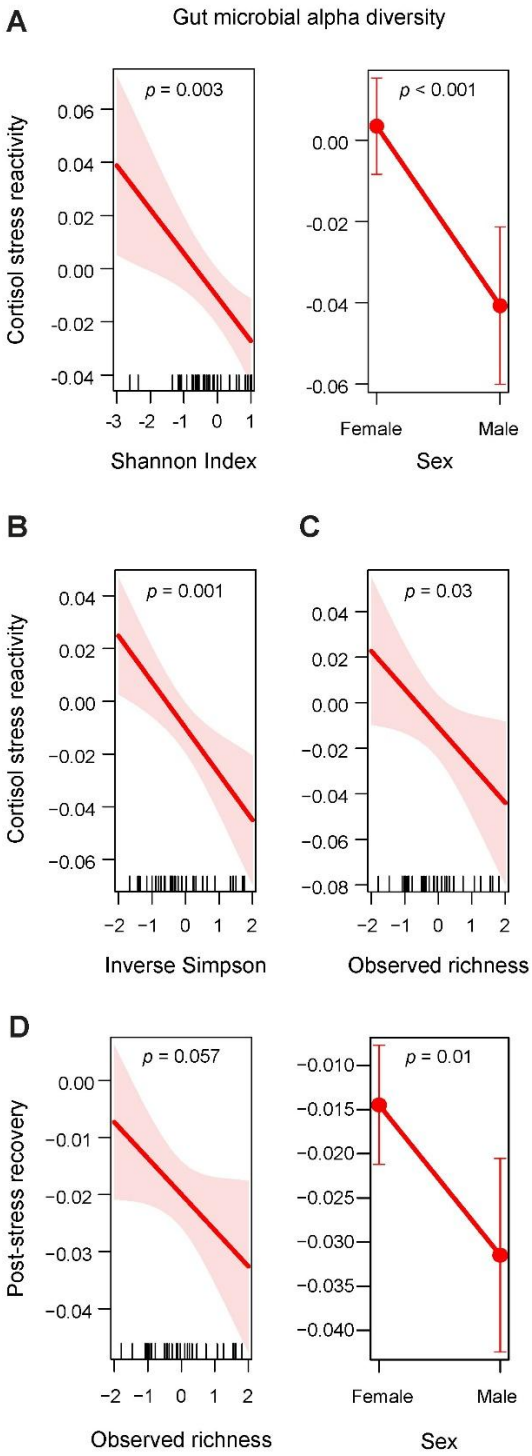


Figure 2. Gut microbial alpha diversity is associated with cortisol stress reactivity. Partial effects from robust linear regression models (RLMs) predicting cortisol stress reactivity (log-transformed nmol/L values) based on the different alpha diversity metrics: **(A)** Shannon Index, **(B)** Inverse Simpson Index, **(C)** Observed number of ASVs (i.e., richness). Male biological sex was significantly associated with lower cortisol stress reactivity across all models (but results are only shown for the Shannon diversity model in panel A; for full model results, see Table 2). **(D)** Observed richness showed a trend-level association with faster post-stress recovery (see Table 3). Shaded areas represent 95% confidence intervals, and rug plots indicate the distribution of observed data points along the predictor axis.

3.3. Gut microbial alpha diversity is related to subjective stress

Because cortisol dynamics in response to acute stress are not necessarily tied to levels of subjective stress (Campbell & Ehlert, 2012), we next evaluated whether the relationship between gut microbial alpha diversity and stress reactivity was also mirrored in terms of subjective stress. To this end, we quantified subjective stress reactivity as the slope of the increase in subjective stress levels following the acute stress intervention for all individuals of the stress group, and repeated the analysis using subjective stress reactivity as the dependent variable (see Methods section for details). The model structure was similar to the above (but note that baseline cortisol at T_2 was not included).

Results showed that higher gut microbial diversity was significantly associated with lower subjective stress reactivity across participants (separate RLMs, N = 36 each). Specifically, higher Shannon diversity was associated with lower subjective stress reactivity (b = -0.033, SE = 0.013, z = -2.439, p = .015; variance-based R^2 = 0.149). Higher Inverse Simpson diversity (b = -0.027, SE = 0.013, z = -2.108, p = .035; variance-based R^2 = 0.119) and a higher number of observed ASVs (b = -0.032, SE = 0.014, z = -2.257, p = .024; variance-based R^2 = 0.145) significantly predicted lower subjective stress reactivity as well. Biological males showed generally lower subjective stress reactivity in the Shannon model (p = .040), and trend-level associations in the remaining two models (ps = .051–.062). General anxiety showed trend-level associations in the Shannon (p = .069) and Inverse Simpson (p = .057) models, but not in the observed richness model (p = .119). Perceived stress was not significantly associated with subjective stress reactivity in any of the models (all $p > 0.22$; see Table 4).

Thus, in line with our results on cortisol stress reactivity, higher gut microbial alpha diversity was significantly associated with lower subjective stress reactivity across participants, suggesting that individuals with a more diverse gut microbiota may exhibit a more attenuated psychophysiological response to acute stress.

3.4. Gut microbial capacity to produce butyrate is associated with cortisol stress reactivity, but not subjective stress

The gut microbiota was shown to influence stress physiology through the production of short-chain fatty acids (SCFAs). Prior evidence highlighted that SCFAs (particularly butyrate, but also propionate and acetate) reduced corticosterone release following acute stress exposure in chronically stressed mice (Van De Wouw et al., 2018), and that they attenuated the cortisol surge after psychological stress in humans (Dalile et al., 2020). We hypothesized that a higher microbial capacity to produce SCFAs would be tied to lower cortisol stress reactivity and faster post-stress recovery. We thus estimated each individual's microbial SCFA-production capacity by identifying bacterial taxa with known butyrate- or propionate-producing capabilities based on their taxonomic classifications (see Methods section for details; see Table 1). We did not include acetate in our analysis, as its biosynthesis pathway is highly abundant and present in almost all gut bacteria (Frolova et al., 2022; Louis & Flint, 2017). We then grouped bacterial taxa according to their capacity to produce butyrate and propionate and calculated the summed relative abundance per participant (thus, yielding estimates of the relative abundance of potential butyrate- and propionate-producing bacteria in each participant's gut microbiome).

Across the participants in the stress group, we identified butyrate-producing bacteria with a relative abundance ranging from 12.2% to 54.9% (mean = 29.7%). These values are consistent with estimates from Frolova et al. (2022), who reported a mean predicted butyrate-producing phenotype abundance of 28.0% (IQR: 21.0–34.3%) in a large twin cohort Frolova et al., 2022). As predicted, results showed that a higher relative abundance of butyrate-producing bacteria was significantly associated with lower cortisol stress reactivity across individuals (b = –

0.0145, SE = 0.0069, z = –2.09, p = .036; variance-based R^2 = 0.144; Figure 3A, Table 5). However, there was no significant association between post-stress recovery (p = .822; variance-based R^2 = 0.258; Figure 3B, Table 5) or subjective stress reactivity (b = –0.0081, SE = 0.0128, z = –0.63, p = .527; variance-based R^2 = 0.074; Figure 3A-B, see supplement, Table S2).

Propionate-producing bacteria showed a relative abundance ranging from 73.1% to 96.9% (mean = 89.2%), which is substantially higher than expected based on the previously reported mean of 35.9% and 75th percentile of 46.2% (Frolova et al., 2022). This discrepancy may reflect differences in the taxonomic classification between studies. The relative abundance of propionate-producing bacteria was not significantly linked to cortisol stress reactivity (b = –0.0100, SE = 0.0761, z = 1.313, p = .188; variance-based R^2 = 0.123; Figure 3C, Table 6). We also could not confirm a significant relationship between the relative abundance of propionate producers and post-stress recovery (p = .643; variance-based R^2 = 0.267; Figure 3D, Table 6), or subjective stress reactivity (b = 0.0078, SE = 0.0130, z = 0.60, p = .549; variance-based R^2 = 0.073; Figure 3C-D, see supplement, Table S2).

To summarize, we found that a higher relative abundance of potential butyrate-producing bacteria was specifically tied to lower cortisol stress reactivity across participants. We could not confirm the link between propionate-production capacity and psychophysiological stress across individuals, which might be due to a limited ability to identify propionate producers with sufficient taxonomic specificity.

Table 4. Robust linear models (Microbiome diversity) predicting subjective stress reactivity

Results from robust linear regression models predicting subjective stress reactivity based on microbiome diversity (Shannon Index/Inverse Simpson/Observed), sex, and psychological variables (general anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$. Model formulas: Subjective stress reactivity ~ biological sex + general anxiety + perceived stress + Shannon/Inverse Simpson/Observed.

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (Shannon) predicting subjective stress reactivity					
(Intercept)	-0.062	0.015	-4.270	0.00002	***
Biological sex	-0.057	0.028	-2.049	0.040	*
General anxiety	0.036	0.020	1.821	0.069	
Perceived Stress	-0.023	0.020	-1.160	0.246	
Shannon	-0.032	0.013	-2.439	0.015	*
RLM (Inverse Simpson) predicting subjective stress reactivity					
(Intercept)	-0.063	0.014	-4.540	0.00001	***
Biological sex	-0.051	0.027	-1.904	0.057	
General anxiety	0.036	0.019	1.906	0.057	
Perceived Stress	-0.023	0.019	-1.210	0.226	
Inverse Simpson	-0.027	0.013	-2.108	0.035	*
RLM (Observed) predicting subjective stress reactivity					
(Intercept)	-0.064	0.015	-4.356	0.00001	***
Biological sex	-0.053	0.028	-1.866	0.062	
General anxiety	0.032	0.020	1.558	0.119	
Perceived Stress	-0.022	0.020	-1.078	0.281	
Observed	-0.032	0.014	-2.257	0.024	*

Table 5. Robust linear models (SCFA Butyrate) predicting cortisol stress reactivity and post-stress recovery

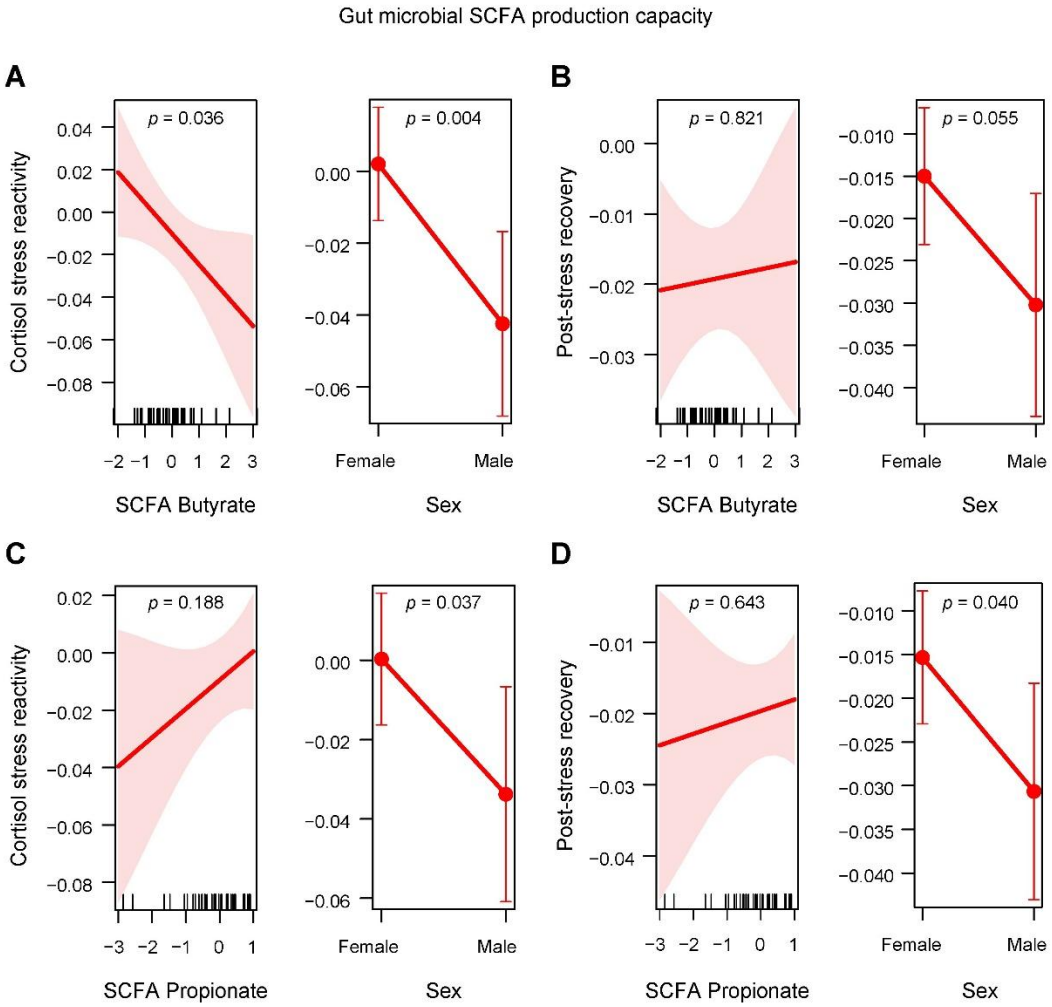
Results from robust linear regression model predicting cortisol stress reactivity and post-stress recovery based on SCFA (butyrate) production capacity, baseline cortisol, sex, and psychological variables (general anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$. Model formulas: Cortisol stress reactivity/Post-stress recovery ~ biological sex + general anxiety + perceived stress + butyrate + baseline cortisol (T_2).

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (SCFA Butyrate) predicting cortisol stress reactivity					
(Intercept)	0.002	0.008	0.252	0.801	
Biological sex	-0.044	0.015	-2.869	0.004	**
General anxiety	-0.004	0.011	-0.360	0.719	
Perceived Stress	0.002	0.011	0.143	0.886	
Butyrate	-0.014	0.007	-2.096	0.036	*
baseline cortisol (T_2)	0.001	0.007	0.118	0.906	
RLM (SCFA Butyrate) predicting post-stress recovery					
(Intercept)	-0.015	0.004	-3.679	0.0002	***
Biological sex	-0.015	0.008	-1.914	0.056	
General anxiety	-0.013	0.006	-2.367	0.018	*
Perceived Stress	0.008	0.006	1.402	0.161	
Butyrate	0.001	0.004	0.225	0.822	
baseline cortisol (T_2)	-0.020	0.004	-5.603	0.00000	***

Table 6. Robust linear models (SCFA Propionate) predicting cortisol stress reactivity and post-stress recovery

Results from robust linear regression models predicting cortisol stress reactivity and post-stress recovery based on SCFA (propionate) production capacity, baseline cortisol, sex, and psychological variables (general anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$. Model formulas: Cortisol stress reactivity/Post-stress recovery ~ biological sex + general anxiety + perceived stress + propionate + baseline cortisol (T_2).

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (SCFA Propionate) predicting cortisol stress reactivity					
(Intercept)	-0.0002	0.008	-0.026	0.979	
Biological sex	-0.034	0.016	-2.082	0.037	*
General anxiety	-0.016	0.012	-1.360	0.174	
Perceived Stress	0.012	0.012	1.033	0.301	
Propionate	0.010	0.008	1.314	0.189	
baseline cortisol (T_2)	-0.001	0.007	-0.169	0.865	
RLM (SCFA Propionate) predicting post-stress recovery					
(Intercept)	-0.016	0.004	-4.031	0.0001	***
Biological sex	-0.015	0.007	-2.047	0.041	*
General anxiety	-0.014	0.005	-2.717	0.007	**
Perceived Stress	0.008	0.005	1.610	0.107	
Propionate	0.002	0.003	0.462	0.644	
baseline cortisol (T_2)	-0.020	0.003	-5.959	0	***



542

543 **Figure 3. Gut microbial SCFA-production capacity is associated with cortisol stress**
544 **reactivity.** Partial effects from robust linear regression models (RLMs) predicting cortisol
545 stress reactivity and post-stress recovery (log-transformed nmol/L values) based on microbial
546 SCFA-production capacity for **(A)** butyrate and **(B)** propionate. **(A)** Higher relative abundance
547 of potential butyrate-producing bacteria was significantly associated with lower cortisol stress
548 reactivity. Male biological sex was also associated with lower reactivity and showed a trend
549 toward faster post-stress recovery. **(B)** Propionate-producing bacteria were not significantly
550 associated with cortisol stress reactivity or post-stress recovery. In the propionate models, male
551 biological sex significantly predicted lower cortisol stress reactivity and faster post-stress
552 recovery. For full model results, see also Tables 5-6. Shaded areas represent 95% confidence
553 intervals, and rug plots indicate the distribution of observed data points along the predictor
554 axis.

555

4. Discussion

In the present study, our goal was to test whether individual differences in gut microbiota composition and in one's microbial capacity to produce SCFAs were linked to cortisol stress reactivity and post-stress recovery following acute stress in healthy adults. Several key findings emerged: First, higher gut microbial alpha diversity was associated with lower cortisol stress reactivity and lower levels of subjective stress across individuals. This was consistently observed for several well-established alpha diversity metrics, highlighting a modulatory role of the gut microbiota on both the physiological and psychological dimensions of stress. Contrary to what we had expected, microbiota diversity was not significantly related to post-stress recovery. Second, individuals with a higher relative abundance of butyrate-producing bacteria tended to show lower cortisol stress reactivity in response to acute stress. Together, these results are the first to highlight the connection between gut microbiota composition, inferred SCFA-production capacity, and psychophysiological stress-response in healthy adults, suggesting that human gut bacteria may help preventing exaggerated stress.

Consistent with our hypothesis that the acute stress response would be linked to gut microbiota composition across individuals, higher alpha diversity was associated with lower cortisol stress reactivity. This association persisted when controlling for confounders (such as baseline cortisol or trait anxiety) and was consistently observed across different alpha diversity metrics. Together, these findings align with prior animal research showing that stress-exposed germ-free mice displayed an exaggerated HPA axis response. This was normalized with probiotic colonization that increased microbial diversity and SCFA production (Sudo et al., 2004). Likewise, probiotic administration reduced corticosterone release and anxiety-like behavior in animals (Bravo et al., 2011). In humans, probiotic supplementation was shown to buffer against the negative effects of acute stress on cognition and mood (Papalini et al., 2019; Steenbergen et al., 2015). Besides cortisol stress reactivity, we found that higher microbial alpha diversity was associated with lower subjective stress reactivity. Put differently, individuals with a more diverse gut microbiota composition exhibited a diminished psychophysiological response to the standardized acute stress intervention. Notably, cortisol and subjective stress are not necessarily correlated (Campbell & Ehlert, 2012; Hellhammer & Schubert, 2012), with some individuals showing elevated cortisol levels without reporting elevated subjective stress or vice versa. We also examined the role of biological sex with respect to the microbiota-stress link, since both the acute stress response and microbiota composition differ depending on biological sex (Adjei et al., 2018; Stefanaki et al., 2022). While we found that biological males exhibited generally lower cortisol stress reactivity and faster post-stress recovery than females, a sex-dependent effect for subjective stress reactivity was observed only in relation to Shannon diversity. To date, only a few studies have explored the microbiota-stress link in humans, focusing on patients with major depression (Kelly et al., 2016) or infants (Rosin et al., 2021). Here, we present the first evidence in healthy adults that underscores the potential buffering role of a diverse gut microbiota in the psychophysiological response to acute stress.

Mechanistically, higher alpha diversity has been proposed to reflect a more stable microbial ecosystem that confers functional resilience through taxonomic redundancy (Lozupone et al., 2012), potentially buffering against perturbations (Shade et al., 2012), such as psychological stress (Foster & Neufeld, 2013; Sheng et al., 2025). Altered microbiota diversity was reported in patients with major depression (Kelly et al., 2016; Zheng et al., 2016), anxiety disorders (Jiang et al., 2015, 2018), and irritable bowel disease (Pittayanon et al., 2019). Reduced alpha diversity can co-occur with increased gut permeability, as shown in alcohol-dependent individuals (Leclercq et al., 2014), facilitating the translocation of bacterial components such as lipopolysaccharides (LPS) into the circulation (Madison & Bailey, 2024). This, in turn, can trigger systemic inflammation and HPA axis activation (Kelly et al., 2015; Madison & Bailey,

605 2024). Thus, by preserving intestinal barrier integrity, a diverse gut microbiota may help to
606 suppress systemic inflammation, keeping neuroinflammation and the activation of stress-
607 related brain circuits in check (Madison & Bailey, 2024). Microbiota depletion in mice
608 disrupted the transcriptomic and metabolic profiles of stress-sensitive brain regions (e.g., the
609 hippocampus and amygdala), resulting in HPA axis overactivation and altered stress
610 responsivity (Tofani et al., 2025). This establishes the gut microbiota as an important facilitator
611 of HPA axis activity. Here, we show for the first time that the gut microbiota is tied to HPA
612 axis activity also in healthy adults.

613 Moreover, higher microbial diversity may enhance an organism's metabolic flexibility (Moya
614 & Ferrer, 2016), and this can foster the production of neuroactive metabolites such as SCFAs
615 (Bourassa et al., 2016; Dalile et al., 2019; Rea et al., 2016). These are produced by the microbial
616 fermentation of dietary fibers, help to preserve intestinal barrier integrity, regulate immune
617 function, and can affect the central nervous system via vagal and epigenetic pathways (Cryan
618 et al., 2019; Dalile et al., 2019; Peng et al., 2009; Smith et al., 2013; Stilling et al., 2016). We
619 specifically focused on butyrate and propionate (we excluded acetate due to its ubiquitous
620 biosynthesis pathway; Frolova et al., 2022; Louis & Flint, 2017) and hypothesized that a higher
621 microbial capacity to produce these SCFAs would be associated with a diminished effect of
622 acute stress across individuals. Consistent with this, results showed that a higher relative
623 abundance of potential butyrate-producing bacteria was correlated with lower cortisol stress
624 reactivity (but not subjective stress reactivity or post-stress recovery). This aligns with previous
625 rodent studies that demonstrated reduced stress-induced corticosterone release after
626 supplementation with SCFA mixtures (Van De Wouw et al., 2018) or butyrate alone (Wang et
627 al., 2023). In humans, SCFA supplementation attenuated cortisol responses to psychosocial
628 stress in a dose-dependent manner (Dalile et al., 2020), although this was not replicated in a
629 follow-up study that gave butyrate alone (Dalile et al., 2024; but note that the divergent results
630 could reflect potential differences between endogenous SCFA production versus exogenous
631 supplementation). Importantly, one's capacity to produce SCFAs appears directly tied to gut
632 microbial diversity (Frolova et al., 2022), as more diverse communities can occupy a broader
633 array of metabolic niches and entertain cross-feeding interactions (Moya & Ferrer, 2016). For
634 example, the butyrate-producing bacteria *Faecalibacterium prausnitzii* and *Roseburia* spp. rely
635 on acetate or lactate from other microbes, illustrating metabolic interdependence within
636 microbial communities. Thus, a diverse and functionally redundant microbial community can
637 likely sustain SCFA production and exert beneficial effects on HPA axis activity.

638 Contrary to what we had expected, the microbial capacity for propionate production was not
639 significantly associated with any of the stress-related parameters. This may reflect a genuine
640 effect of differential actions when comparing propionate and butyrate, as prior works reported
641 mixed results. While some reported beneficial effects of propionate supplementation on stress
642 physiology (Dalile et al., 2020; Van De Wouw et al., 2018), others noted no significant, or even
643 anxiogenic, effects at higher propionate concentrations (Choi et al., 2018; Kimura et al., 2011).
644 SCFAs can exert distinct and even opposing effects on host physiology (Kimura et al., 2011).
645 Therefore, considering interactions between different SCFAs might be of relevance. Their
646 stress-related actions may depend on the exact mixture and dosage of supplementation, the type
647 of physiological system that is primarily modulated (e.g., immune-, endocrine-, or neural
648 systems), or host specifics (e.g., interindividual differences in microbiota composition, or stress
649 sensitivity; Dalile et al., 2020; Rosell-Cardona et al., 2025). Together, these findings position
650 SCFAs, particularly butyrate, as key microbial metabolites that link the gut microbiota with
651 HPA axis regulation. While our results highlight that individual differences in inferred,
652 endogenous butyrate-production capacity are associated with attenuated cortisol stress
653 reactivity in healthy adults, they do not preclude that other SCFAs (e.g., propionate or acetate)

may also contribute to acute stress modulation. Future studies should consider pathway-level functional annotation or metabolomics to clarify the distinct and joint roles of specific SCFAs in shaping the stress response.

More specifically, SCFAs can modulate HPA axis activity via several pathways. This includes direct action by binding to free fatty acid receptors (FFAR2/3) on enteroendocrine and immune cells (Maslowski et al., 2009; Tedelind et al., 2007), as well as to vagal afferents that relay microbial signals to stress-related brain regions (De Vadder et al., 2014; Fülling et al., 2019; Kimura et al., 2011). SCFAs can further cross the blood-brain barrier and inhibit histone deacetylases, affecting stress-related gene expression (Lupori et al., 2022; Stilling et al., 2016). Indirectly, SCFAs were shown to reduce inflammation via pro-inflammatory pathways, T-cells, and microglia (Silva et al., 2020; Smith et al., 2013; Wenzel et al., 2020). Butyrate in particular enhances tight junctions and preserves barrier integrity by preventing LPS translocation under stress (Braniste et al., 2014; Kelly et al., 2015; Rosell-Cardona et al., 2025). Consistent with this, greater microbial butyrate-production capacity was associated with lower cortisol stress reactivity. While we are unable to pinpoint the specific underlying mechanisms, we suggest that butyrate producers may influence the psychophysiological response to acute stress via multiple mechanisms, from supporting barrier integrity, the regulation of neuroinflammation, to direct interactions with the HPA-axis.

We would also like to discuss several limitations of our approach. First, we inferred individuals' SCFA-production capacity from 16S rRNA gene amplicon sequencing data rather than directly measuring SCFAs concentrations. However, Frolova et al. (2022) found that comparable data of SCFA production capacity was significantly correlated with SCFA levels obtained from an *in vitro* assay, specifically butyrate, supporting proxy validity. The sequencing approach might have also limited us in adequately identifying the abundance of potential propionate producers (note that we identified a substantially higher abundance than prior work, limiting the specificity of our results). Second, we could not confirm a link between gut microbiota diversity and post-stress recovery, but note that higher microbiota richness showed a clear trend-level association with faster post-stress recovery. While this could reflect a true null effect, the result could also be affected by insufficient power for detecting post-stress recovery effects, given the relatively small group size of the stress group (n=36). However, prior studies with similar sample sizes reported robust microbiota-stress associations (e.g., Delgadillo et al., 2025; Kato-Kataoka et al., 2016; Schmidt et al., 2015). Alternatively, a true effect on post-stress recovery may have been masked by our stress reminder (i.e., to ensure a substantial effect of the acute stress intervention, we repeated part of the stressful procedure after a delay). Future studies should use larger samples and integrate recovery-relevant behavioral, microbial, and physiological markers.

To conclude, we investigated the role of the gut microbiota in modulating the acute stress response in healthy adults. Greater microbial diversity was associated with lower cortisol stress reactivity and lower subjective stress reactivity after an effective stress intervention. Importantly, cortisol stress reactivity was also tied to the inferred capacity for producing the key SCFA butyrate, with higher production capacity going hand in hand with lower cortisol stress reactivity. These results are the first to highlight the role of gut microbial diversity and butyrate-production capacity in modulating stress reactivity in healthy adults, underscoring the microbiota's potential to buffer against the detrimental effects of acute stress.

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6. Author contributions

Conceptualization: T.K., I.C.W.; Data curation: T.K., I.C.W.; Formal analysis: T.K., I.C.W.; Funding acquisition: I.C.W.; Investigation: T.K., I.C.W.; Methodology: all authors; Project administration: T.K., I.C.W.; Resources: I.C.W.; Software: T.K., I.C.W.; Supervision: I.C.W.; Validation: T.K., I.C.W.; Visualization: T.K., I.C.W.; Writing – original draft: T.K., I.C.W.; Writing – reviewing and editing: all authors.

7. Conflict of Interest

The authors declare no conflict of interest.

8. References

Adjei, T., Xue, J., & Mandic, D. P. (2018). The Female Heart: Sex Differences in the Dynamics of ECG in Response to Stress. *Frontiers in Physiology*, *9*, 1616. https://doi.org/10.3389/fphys.2018.01616

Alboukadel Kassambara. (2021). *rstatix: Pipe-Friendly Framework for Basic Statistical Tests* [Computer software]. R package. https://CRAN.R-project.org/package=rstatix

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., & Albertsen, M. (2018). *ampvis2: An R package to analyse and visualise 16S rRNA amplicon data*. Bioinformatics. https://doi.org/10.1101/299537

Apprill, A., Mcnally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/AME01753

Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, *68*(3), 255–278. https://doi.org/10.1016/j.jml.2012.11.001

Bourassa, M. W., Alim, I., Bultman, S. J., & Ratan, R. R. (2016). Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neuroscience Letters*, *625*, 56–63. https://doi.org/10.1016/j.neulet.2016.02.009

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Tóth, M., Korecka, A., Bakocevic, N., Ng, L. G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B. T., Diamond, B., & Pettersson, S. (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Science Translational Medicine*, *6*(263), 263ra158-263ra158. https://doi.org/10.1126/scitranslmed.3009759

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*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods 2016 13:7*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Campbell, J., & Ehlert, U. (2012). Acute psychosocial stress: Does the emotional stress response correspond with physiological responses? *Psychoneuroendocrinology*, *37*(8), 1111–1134. https://doi.org/10.1016/j.psyneuen.2011.12.010

Casterline, J. L., Oles, C. J., & Ku, Y. (1997). *In Vitro* Fermentation of Various Food Fiber Fractions. *Journal of Agricultural and Food Chemistry*, *45*(7), 2463–2467. https://doi.org/10.1021/jf960846f

Choi, J., Lee, S., Won, J., Jin, Y., Hong, Y., Hur, T.-Y., Kim, J.-H., Lee, S.-R., & Hong, Y. (2018). Pathophysiological and neurobehavioral characteristics of a propionic acid-mediated autism-like rat model. *PLOS ONE*, *13*(2), e0192925. https://doi.org/10.1371/journal.pone.0192925

Cohen, S. (1988). Perceived stress in a probability sample of the United States. In *The social psychology of health* (pp. 31–67). Sage Publications, Inc.

Cryan, J. F., O'riordan, K. J., Cowan, C. S. M., Sandhu, K. 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., … Dinan, T. G. (2019). The microbiota-gut-brain axis. *Physiological Reviews*, *99*(4), 1877–2013. https://doi.org/10.1152/PHYSREV.00018.2018

Dalile, B., Fuchs, A., La Torre, D., Vervliet, B., Van Oudenhove, L., & Verbeke, K. (2024). Colonic butyrate administration modulates fear memory but not the acute stress response in men: A randomized, triple-blind, placebo-controlled trial. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *131*, 110939. https://doi.org/10.1016/j.pnpbp.2024.110939

Dalile, B., Van Oudenhove, L., Vervliet, B., & Verbeke, K. (2019). The role of short-chain fatty acids in microbiota–gut–brain communication. *Nature Reviews Gastroenterology & Hepatology*, *16*(8), 461–478. https://doi.org/10.1038/s41575-019-0157-3

Dalile, B., Vervliet, B., Bergonzelli, G., Verbeke, K., & Van Oudenhove, L. (2020). Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: A randomized, placebo-controlled trial. *Neuropsychopharmacology*, *45*(13), 2257–2266. https://doi.org/10.1038/s41386-020-0732-x

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods*, *8*(3), 92. https://doi.org/10.3390/foods8030092

Davis, N. M., Proctor, Di. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 1–14. https://doi.org/10.1186/S40168-018-0605-2/FIGURES/6

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463–475. https://doi.org/10.1038/nrn1683

Dedovic, K., Renwick, R., Khalili Mahani, N., Engert, V., Lupien, S. J., Pruessner, J. C., Mahani, K., & -Douglas, P. (2005). The Montreal Imaging Stress Task: Using

functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. In *J Psychiatry Neurosci* (Vol. 30, Issue 5).

Delgadillo, D. R., Borelli, J. L., Mayer, E. A., Labus, J. S., Cross, M. P., & Pressman, S. D. (2025). Biological, environmental, and psychological stress and the human gut microbiome in healthy adults. *Scientific Reports*, *15*(1), 362. https://doi.org/10.1038/s41598-024-77473-9

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., Bäckhed, F., & Mithieux, G. (2014). Microbiota-Generated Metabolites Promote Metabolic Benefits via Gut-Brain Neural Circuits. *Cell*, *156*(1), 84–96. https://doi.org/10.1016/j.cell.2013.12.016

Dickerson, S. S., & Kemeny, M. E. (2004). Acute Stressors and Cortisol Responses: A Theoretical Integration and Synthesis of Laboratory Research. *Psychological Bulletin*, *130*(3), 355–391. https://doi.org/10.1037/0033-2909.130.3.355

Doeller, C. F., Barry, C., & Burgess, N. (2010). Evidence for grid cells in a human memory network. *Nature*, *463*(7281), 657–661. https://doi.org/10.1038/nature08704

Fiksdal, A., Hanlin, L., Kuras, Y., Gianferante, D., Chen, X., Thoma, M. V., & Rohleder, N. (2019). Associations between symptoms of depression and anxiety and cortisol responses to and recovery from acute stress. *Psychoneuroendocrinology*, *102*, 44–52. https://doi.org/10.1016/j.psyneuen.2018.11.035

Forbes, P. A. G., Aydogan, G., Braunstein, J., Todorova, B., Wagner, I. C., Lockwood, P. L., Apps, M. A. J., Ruff, C. C., & Lamm, C. (2024). Acute stress reduces effortful prosocial behaviour. *eLife*, *12*, RP87271. https://doi.org/10.7554/eLife.87271

Foster, J. A., & Neufeld, K.-A. M. (2013). Gut–brain axis: How the microbiome influences anxiety and depression. *Trends in Neurosciences*, *36*(5), 305–312. https://doi.org/10.1016/j.tins.2013.01.005

Foster, J. A., Rinaman, L., & Cryan, J. F. (2017). Stress & the gut-brain axis: Regulation by the microbiome. *Neurobiology of Stress*, *7*, 124–136. https://doi.org/10.1016/j.ynstr.2017.03.001

Fox, J., & Weisberg, S. (2018). Visualizing Fit and Lack of Fit in Complex Regression Models with Predictor Effect Plots and Partial Residuals. *Journal of Statistical Software*, *87*(9). https://doi.org/10.18637/jss.v087.i09

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (3rd ed.). Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/index.html

Frolova, M. S., Suvorova, I. A., Iablokov, S. N., Petrov, S. N., & Rodionov, D. A. (2022). Genomic reconstruction of short-chain fatty acid production by the human gut microbiota. *Frontiers in Molecular Biosciences*, *9*, 949563. https://doi.org/10.3389/fmolb.2022.949563

Fülling, C., Dinan, T. G., & Cryan, J. F. (2019). Gut Microbe to Brain Signaling: What Happens in Vagus…. *Neuron*, *101*(6), 998–1002. https://doi.org/10.1016/j.neuron.2019.02.008

Grimaldi, R., Cela, D., Swann, J. R., Vulevic, J., Gibson, G. R., Tzortzis, G., & Costabile, A. (2017). In vitro fermentation of B-GOS: Impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. *FEMS Microbiology Ecology*, *93*(2), fiw233. https://doi.org/10.1093/femsec/fiw233

Hadley, W. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag.

Hellhammer, J., & Schubert, M. (2012). The physiological response to Trier Social Stress Test relates to subjective measures of stress during but not before or after the test. *Psychoneuroendocrinology*, *37*(1), 119–124. https://doi.org/10.1016/j.psyneuen.2011.05.012

Herbold, C. W., Pelikan, C., Kuzyk, O., Hausmann, B., Angel, R., Berry, D., & Loy, A. (2015). A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. *Frontiers in Microbiology*, *6*(JUL), 731. https://doi.org/10.3389/FMICB.2015.00731/BIBTEX

Hlavac, M. (2022). *stargazer: Well-Formatted Regression and Summary Statistics Tables*. Social Policy Institute. https://CRAN.R-project.org/package=stargazer

Huber, W., Carey, V. J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B. S., Bravo, H. C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K. D., Irizarry, R. A., Lawrence, M., Love, M. I., MacDonald, J., Obenchain, V., Oleś, A. K., … Morgan, M. (2015). Orchestrating high-throughput genomic analysis with Bioconductor. *Nature Methods*, *12*(2), 115–121. https://doi.org/10.1038/nmeth.3252

Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., & Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity*, *48*, 186–194. https://doi.org/10.1016/j.bbi.2015.03.016

Jiang, H., Zhang, X., Yu, Z., Zhang, Z., Deng, M., Zhao, J., & Ruan, B. (2018). Altered gut microbiota profile in patients with generalized anxiety disorder. *Journal of Psychiatric Research*, *104*, 130–136. https://doi.org/10.1016/j.jpsychires.2018.07.007

Johnson, A. J., Zheng, J. J., Kang, J. W., Saboe, A., Knights, D., & Zivkovic, A. M. (2020). A Guide to Diet-Microbiome Study Design. *Frontiers in Nutrition*, *7*, 79. https://doi.org/10.3389/fnut.2020.00079

Kato-Kataoka, A., Nishida, K., Takada, M., Suda, K., Kawai, M., Shimizu, K., Kushiro, A., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Kuwano, Y., & Rokutan, K. (2016). *Fermented milk containing Lactobacillus casei strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress*. https://doi.org/10.3920/BM2015.0100

Kelly, J. R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P. J., Beers, S., Scott, K., Moloney, G., Hoban, A. E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J. F., & Dinan, T. G. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research*, *82*, 109–118. https://doi.org/10.1016/j.jpsychires.2016.07.019

Kelly, J. R., Kennedy, P. J., Cryan, J. F., Dinan, T. G., Clarke, G., & Hyland, N. P. (2015). Breaking down the barriers: The gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Frontiers in Cellular Neuroscience*, *9*. https://doi.org/10.3389/fncel.2015.00392

Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., Kobayashi, M., Hirasawa, A., & Tsujimoto, G. (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proceedings of the National Academy of Sciences*, *108*(19), 8030–8035. https://doi.org/10.1073/pnas.1016088108

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*, *19*(4), 313–333. https://doi.org/10.1016/0306-4530(94)90013-2

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). *Impact of Gender, Menstrual Cycle Phase, and Oral Contraceptives on the Activity of the Hypothalamus-Pituitary-Adrenal Axis*.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier social stress test'—A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81. https://doi.org/10.1159/000119004

Lach, G., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2018). Anxiety, Depression, and the Microbiome: A Role for Gut Peptides. *Neurotherapeutics*, *15*(1), 36–59. https://doi.org/10.1007/s13311-017-0585-0

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(NOV). https://doi.org/10.3389/fpsyg.2013.00863

Leclercq, S., Matamoros, S., Cani, P. D., Neyrinck, A. M., Jamar, F., Stärkel, P., Windey, K., Tremaroli, V., Bäckhed, F., Verbeke, K., De Timary, P., & Delzenne, N. M. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proceedings of the National Academy of Sciences*, *111*(42). https://doi.org/10.1073/pnas.1415174111

Lewis, S. J., & and Heaton, K. W. (1997). Stool Form Scale as a Useful Guide to Intestinal Transit Time. *Scandinavian Journal of Gastroenterology*, *32*(9), 920–924. https://doi.org/10.3109/00365529709011203

Linz, R., Puhlmann, L. M. C., Apostolakou, F., Mantzou, E., Papassotiriou, I., Chrousos, G. P., Engert, V., & Singer, T. (2019). Acute psychosocial stress increases serum BDNF levels: An antagonistic relation to cortisol but no group differences after mental training. *Neuropsychopharmacology*, *44*(10), 1797–1804. https://doi.org/10.1038/s41386-019-0391-y

Louis, P., & Flint, H. J. (2017). Formation of propionate and butyrate by the human colonic microbiota. *Environmental Microbiology*, *19*(1), 29–41. https://doi.org/10.1111/1462-2920.13589

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220–230. https://doi.org/10.1038/nature11550

Lupori, L., Cornuti, S., Mazziotti, R., Borghi, E., Ottaviano, E., Cas, M. D., Sagona, G., Pizzorusso, T., & Tognini, P. (2022). The gut microbiota of environmentally enriched mice regulates visual cortical plasticity. *Cell Reports*, *38*(2), 110212. https://doi.org/10.1016/j.celrep.2021.110212

Madison, A. A., & Bailey, M. T. (2024). Stressed to the Core: Inflammation and Intestinal Permeability Link Stress-Related Gut Microbiota Shifts to Mental Health Outcomes. *Biological Psychiatry*, *95*(4), 339–347. https://doi.org/10.1016/j.biopsych.2023.10.014

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., Schilter, H. C., Rolph, M. S., Mackay, F., Artis, D., Xavier, R. J., Teixeira, M. M., & Mackay, C. R. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*, *461*(7268), 1282–1286. https://doi.org/10.1038/nature08530

McEwen, B. S. (1993). Stress and the Individual: Mechanisms Leading to Disease. *Archives of Internal Medicine*, *153*(18), 2093. https://doi.org/10.1001/archinte.1993.00410180039004

McEwen, B. S. (2017). Neurobiological and Systemic Effects of Chronic Stress. *Chronic Stress*, *1*, 2470547017692328. https://doi.org/10.1177/2470547017692328

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Miller, R., Plessow, F., Kirschbaum, C., & Stalder, T. (2013). Classification Criteria for Distinguishing Cortisol Responders From Nonresponders to Psychosocial Stress: Evaluation of Salivary Cortisol Pulse Detection in Panel Designs. *Psychosomatic Medicine*, *75*(9), 832–840. https://doi.org/10.1097/PSY.0000000000000002

Moya, A., & Ferrer, M. (2016). Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance. *Trends in Microbiology*, *24*(5), 402–413. https://doi.org/10.1016/j.tim.2016.02.002

Nielsen, S. E., Segal, S. K., Worden, I. V., Yim, I. S., & Cahill, L. (2013). Hormonal contraception use alters stress responses and emotional memory. *Biological Psychology*, *92*(2), 257–266. https://doi.org/10.1016/j.biopsycho.2012.10.007

O'Riordan, K. J., Collins, M. K., Moloney, G. M., Knox, E. G., Aburto, M. R., Fülling, C., Morley, S. J., Clarke, G., Schellekens, H., & Cryan, J. F. (2022). Short chain fatty acids: Microbial metabolites for gut-brain axis signalling. *Molecular and Cellular Endocrinology*, *546*, 111572. https://doi.org/10.1016/J.MCE.2022.111572

Palleja, A., Mikkelsen, K. H., Forslund, S. K., Kashani, A., Allin, K. H., Nielsen, T., Hansen, T. H., Liang, S., Feng, Q., Zhang, C., Pyl, P. T., Coelho, L. P., Yang, H., Wang, J., Typas, A., Nielsen, M. F., Nielsen, H. B., Bork, P., Wang, J., … Pedersen, O. (2018). Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nature Microbiology*, *3*(11), 1255–1265. https://doi.org/10.1038/s41564-018-0257-9

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. https://doi.org/10.1016/j.ynstr.2018.100141

Peng, L., Li, Z.-R., Green, R. S., Holzmanr, I. R., & Lin, J. (2009). Butyrate Enhances the Intestinal Barrier by Facilitating Tight Junction Assembly via Activation of AMP-Activated Protein Kinase in Caco-2 Cell Monolayers12. *The Journal of Nutrition*, *139*(9), 1619–1625. https://doi.org/10.3945/jn.109.104638

Pittayanon, R., Lau, J. T., Yuan, Y., Leontiadis, G. I., Tse, F., Surette, M., & Moayyedi, P. (2019). Gut Microbiota in Patients With Irritable Bowel Syndrome—A Systematic Review. *Gastroenterology*, *157*(1), 97–108. https://doi.org/10.1053/j.gastro.2019.03.049

Pjevac, P., Hausmann, B., Schwarz, J., Kohl, G., Herbold, C. W., Loy, A., & Berry, D. (2021). An Economical and Flexible Dual Barcoding, Two-Step PCR Approach for Highly Multiplexed Amplicon Sequencing. *Frontiers in Microbiology*, *12*, 669776. https://doi.org/10.3389/fmicb.2021.669776

Pruesse, E., Peplies, J., & Glöckner, F. O. (2012). SINA: Accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics*, *28*(14), 1823–1829. https://doi.org/10.1093/BIOINFORMATICS/BTS252

Pusceddu, M. M., El Aidy, S., Crispie, F., O'Sullivan, O., Cotter, P., Stanton, C., Kelly, P., Cryan, J. F., & Dinan, T. G. (2015). N-3 Polyunsaturated Fatty Acids (PUFAs) Reverse the Impact of Early-Life Stress on the Gut Microbiota. *PLoS ONE*, *10*(10), e0139721. https://doi.org/10.1371/journal.pone.0139721

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(Database issue). https://doi.org/10.1093/NAR/GKS1219

Quigley, E. M. M. (2017). Gut microbiome as a clinical tool in gastrointestinal disease management: Are we there yet? *Nature Reviews Gastroenterology & Hepatology*, *14*(5), 315–320. https://doi.org/10.1038/nrgastro.2017.29

R Core Team. (2024a). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team. (2024b). *The R Stats Package*. R Foundation for Statistical Computing. https://stat.ethz.ch/R-manual/R-devel/library/stats/html/00Index.html

Rastall, R. A., & Gibson, G. R. (2015). Recent developments in prebiotics to selectively impact beneficial microbes and promote intestinal health. *Current Opinion in Biotechnology*, *32*, 42–46. https://doi.org/10.1016/j.copbio.2014.11.002

Rea, K., Dinan, T. G., & Cryan, J. F. (2016). The microbiome: A key regulator of stress and neuroinflammation. *Neurobiology of Stress*, *4*, 23–33. https://doi.org/10.1016/j.ynstr.2016.03.001

Rosell-Cardona, C., Leigh, S.-J., Knox, E., Tirelli, E., Lyte, J. M., Goodson, M. S., Kelley-Loughnane, N., Aburto, M. R., Cryan, J. F., & Clarke, G. (2025). Acute Stress-induced Alterations in Short-Chain Fatty Acids: Implications for the Intestinal and Blood Brain Barriers. *Brain, Behavior, & Immunity - Health*, 100992. https://doi.org/10.1016/j.bbih.2025.100992

Rosin, S., Xia, K., Azcarate-Peril, M. A., Carlson, A. L., Propper, C. B., Thompson, A. L., Grewen, K., & Knickmeyer, R. C. (2021). A preliminary study of gut microbiome variation and HPA axis reactivity in healthy infants. *Psychoneuroendocrinology*, *124*, 105046. https://doi.org/10.1016/j.psyneuen.2020.105046

Sapolsky, R. M. (2019). Stress, Health and Social Behavior. In *Encyclopedia of Animal Behavior* (pp. 163–170). Elsevier. https://doi.org/10.1016/B978-0-12-809633-8.20762-0

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. *Endocrine Reviews*, *21*(1), 55–89. https://doi.org/10.1210/edrv.21.1.0389

Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., & Wüst, S. (2008). Covariance Between Psychological and Endocrine Responses to Pharmacological Challenge and Psychosocial Stress: A Question of Timing. *Biopsychosocial Science and Medicine*, *70*(7), 787. https://doi.org/10.1097/PSY.0b013e3181810658

Schmidt, K., Cowen, P. J., Harmer, C. J., Tzortzis, G., Errington, S., & Burnet, P. W. J. (2015). Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology*, *232*(10), 1793–1801. https://doi.org/10.1007/s00213-014-3810-0

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Bürgmann, H., Huber, D. H., Langenheder, S., Lennon, J. T., Martiny, J. B. H., Matulich, K. L., Schmidt, T. M., & Handelsman, J. (2012). Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology*, *3*(DEC). https://doi.org/10.3389/fmicb.2012.00417

Sheng, X., Jin, L., Yao, Z., Gu, J., Zhu, L., Huang, A., Peng, J., Xu, X., Ge, X., Zhou, W., Sheng, J., Xu, Z., & Bai, R. (2025). Psychological stress-induced systemic corticosterone directly sabotages intestinal stem cells and exacerbates colitis. *Cell Discovery*, *11*(1), 1–14. https://doi.org/10.1038/s41421-025-00796-y

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.00025

Slavin, J. (2013). Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients*, *5*(4), Article 4. https://doi.org/10.3390/nu5041417

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., Glickman, J. N., & Garrett, W. S. (2013). The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Science*, *341*(6145), 569–573. https://doi.org/10.1126/science.1241165

Steenbergen, L., Sellaro, R., Van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity*, *48*, 258–264. https://doi.org/10.1016/j.bbi.2015.04.003

Stefanaki, C., Bacopoulou, F., & Chrousos, G. P. (2022). Gut Microsex/Genderome, Immunity and the Stress Response in the Sexes: An Updated Review. *Sexes*, *3*(4), 533–545. https://doi.org/10.3390/sexes3040039

Stilling, R. M., van de Wouw, M., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2016). The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochemistry International*, *99*, 110–132. https://doi.org/10.1016/j.neuint.2016.06.011

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X., Kubo, C., & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *The Journal of Physiology*, *558*(1), 263–275. https://doi.org/10.1113/jphysiol.2004.063388

Tedelind, S., Westberg, F., Kjerrulf, M., & Vidal, A. (2007). Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: A study with relevance to inflammatory bowel disease. *World Journal of Gastroenterology*, *13*(20), 2826–2832. https://doi.org/10.3748/wjg.v13.i20.2826

Tofani, G. S. S., Leigh, S.-J., Gheorghe, C. E., Bastiaanssen, T. F. S., Wilmes, L., Sen, P., Clarke, G., & Cryan, J. F. (2025). Gut microbiota regulates stress responsivity via the circadian system. *Cell Metabolism*, *37*(1), 138-153.e5. https://doi.org/10.1016/j.cmet.2024.10.003

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. *The Journal of Physiology*, *596*(20), 4923–4944. https://doi.org/10.1113/JP276431

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed). Springer.

Vich Vila, A., Collij, V., Sanna, S., Sinha, T., Imhann, F., Bourgonje, A. R., Mujagic, Z., Jonkers, D. M. A. E., Masclee, A. A. M., Fu, J., Kurilshikov, A., Wijmenga, C., Zhernakova, A., & Weersma, R. K. (2020). Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nature Communications*, *11*(1), 362. https://doi.org/10.1038/s41467-019-14177-z

Vogel, S., Klumpers, F., Krugers, H. J., Fang, Z., Oplaat, K. T., Oitzl, M. S., Joëls, M., & Fernández, G. (2015). Blocking the Mineralocorticoid Receptor in Humans Prevents the Stress-Induced Enhancement of Centromedial Amygdala Connectivity with the Dorsal Striatum. *Neuropsychopharmacology*, *40*(4), 947–956. https://doi.org/10.1038/npp.2014.271

Wang, P., Wu, P.-F., Wang, H.-J., Liao, F., Wang, F., & Chen, J.-G. (2023). Gut microbiome-derived ammonia modulates stress vulnerability in the host. *Nature Metabolism*, *5*(11), 1986–2001. https://doi.org/10.1038/s42255-023-00909-5

Wenzel, T. J., Gates, E. J., Ranger, A. L., & Klegeris, A. (2020). Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. *Molecular and Cellular Neuroscience*, *105*, 103493. https://doi.org/10.1016/j.mcn.2020.103493

Wickham, H., Averick, M., Bryan, J., Chang, W., D', L., Mcgowan, A., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Lin Pedersen, T., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/JOSS.01686

Williams, N. (2014). The GAD-7 questionnaire. *Occupational Medicine*, *64*(3), 224. https://doi.org/10.1093/occmed/kqt161

World Medical Association. (2013). World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA*, *310*(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

Yang, L. L., Millischer, V., Rodin, S., MacFabe, D. F., Villaescusa, J. C., & Lavebratt, C. (2020). Enteric short-chain fatty acids promote proliferation of human neural progenitor cells. *Journal of Neurochemistry*, *154*(6), 635–646. https://doi.org/10.1111/jnc.14928

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222–227. https://doi.org/10.1038/nature11053

Zeileis, A., & Hothorn, T. (2002). Diagnostic Checking in Regression Relationships. *R News*, *2*(3), 7–10.

Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N. D., Licinio, J., Wei, H.,

& Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Molecular Psychiatry*, *21*(6), 786–796. https://doi.org/10.1038/mp.2016.44

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., Kotler, E., Zur, M., Regev-Lehavi, D., Brik, R. B.-Z., Federici, S., Cohen, Y., Linevsky, R., Rothschild, D., Moor, A. E., Ben-Moshe, S., Harmelin, A., Itzkovitz, S., Maharshak, N., … Elinav, E. (2018). Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell*, *174*(6), 1388-1405.e21. https://doi.org/10.1016/j.cell.2018.08.041

Zorn, J. V., Schür, R. R., Boks, M. P., Kahn, R. S., Joëls, M., & Vinkers, C. H. (2017). Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *77*, 25–36. https://doi.org/10.1016/j.psyneuen.2016.11.036

1113 # 9. Supplementary Tables:

1114 **Table S1. SCFA production pathways and associated taxa adapted from Frolova et al.**
1115 **(2022), Table 1. Frontiers in Molecular Biosciences, licensed under CC-BY 4.0.**

1116 Overview of fermentation pathway variants for butyrate and propionate production in the
1117 human gut microbiota reference genomes, including the number of genomes and species per
1118 pathway and top associated taxa (table adapted from Frolova et al., 2022).

1119

Fermentation product	Pathway variants†	# Pathways	Top taxonomic groups
Butyrate	P1	1	Clostridiaceae, Eubacteriaceae, Lachnospiraceae, Streptococcaceae
	P1+P2	2	Clostridiales
	P1+P3	2	Clostridiales, Acidaminococcaceae, Peptoniphilaceae
	P1+P4	2	Clostridiales, Butyricimonas, Odoribacter
	P1+P2+P3	3	Clostridium, Lachnoclostridium
	P1+P2+P4	13	Porphyromonas, Clostridiales
	P2+P4	2	Paraclostridium
	P1+P3+P4	3	Fusobacterium
	P1+P2+P3+P4	4	Fusobacterium
	P2	1	Lachnoclostridium, Porphyromonas, Tannerella
	P4	1	Alistipes, Micromonospora
Propionate	P1	1	Bacteroidetes, Firmicutes, Akkermansia, Enterobacteria, Actinobacteria (Propionibacteria/Corynebacteria)
	P1+P2	2	Peptostreptococcaceae
	P1+P3	2	Enterobacteria, Veillonellaceae
	P1+P2+P3	3	Intestinimonas
	P2	1	Clostridiaceae, Lachnospiraceae, Peptostreptococcaceae
	P2+P3	2	Clostridiaceae, Eubacteriaceae, Peptostreptococcaceae
	P3	1	Diverse Clostridia, Peptoniphilus, Veillonella, Lactobacillus, Enterococcus, Listeria, Enterobacteria, Fusobacterium

1120 † Butyrate pathways: P1 (Acetyl-CoA), P2 (succinate), P3 (glutamate), P4 (lysine); Propionate pathways: P1 (succinate), P2
1121 (lactate), P3 (propanediol).

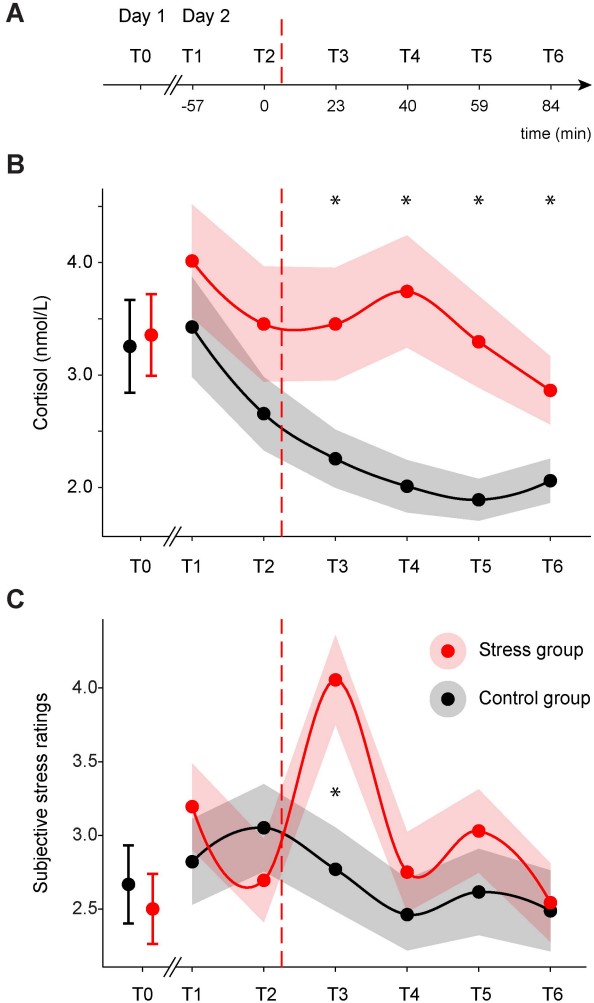
1122 **Table S2. Robust linear models (SCFAs) predicting subjective stress reactivity**

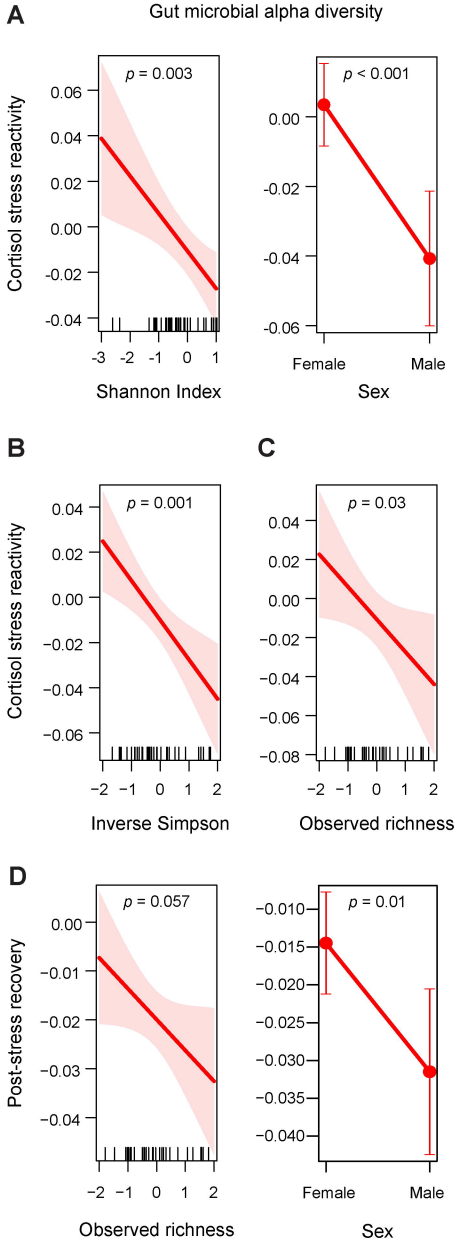
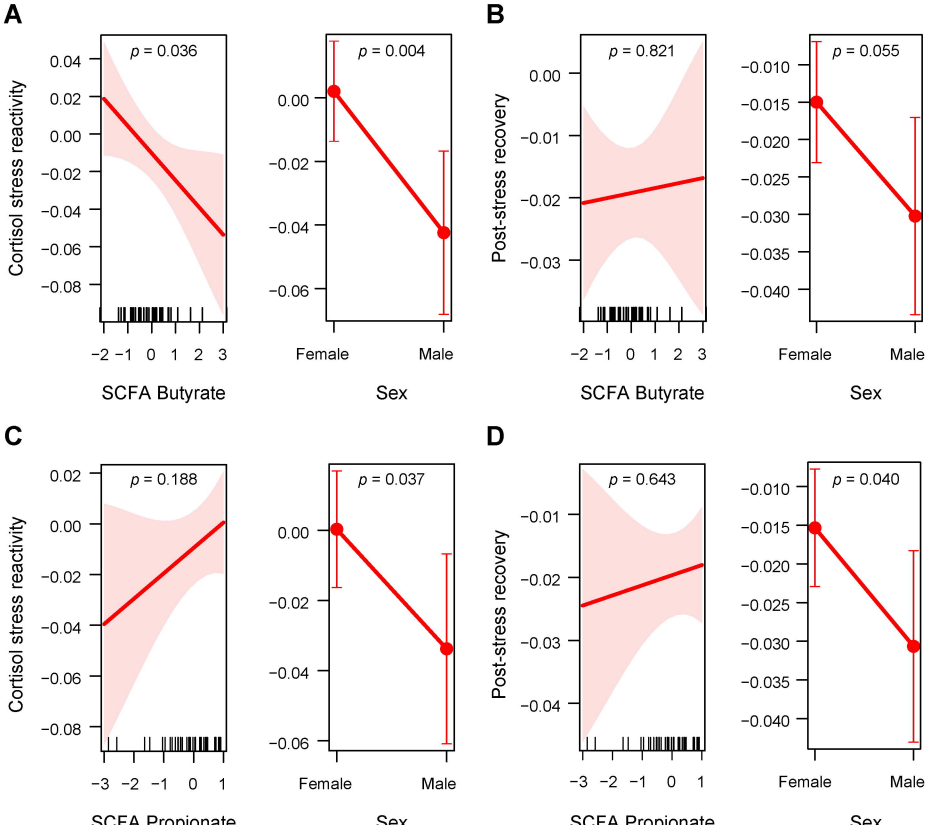
1123 Results from robust linear regression models predicting subjective stress reactivity based on
1124 SCFAs (butyrate/propionate) production capacity, sex, and psychological variables (general
1125 anxiety and perceived stress). * denotes $p < .05$, ** denotes $p < .005$, *** denotes $p < .0001$.
1126 Model formulas: Subjective stress reactivity ~ biological sex + general anxiety + perceived
1127 stress + butyrate/propionate.

1128

Predictor	*b*	SE	*z*	*p*	Sig.
RLM (SCFA Butyrate) predicting subjective stress reactivity					
(Intercept)	-0.064	0.015	-4.316	0.00002	***
Biological sex	-0.039	0.029	-1.362	0.173	
General anxiety	0.043	0.020	2.145	0.032	*
Perceived Stress	-0.032	0.021	-1.540	0.124	
Butyrate	-0.008	0.013	-0.632	0.527	
RLM (SCFA Propionate) predicting subjective stress reactivity					
(Intercept)	-0.065	0.015	-4.422	0.00001	***
Biological sex	-0.041	0.028	-1.451	0.147	
General anxiety	0.041	0.020	2.044	0.041	*
Perceived Stress	-0.029	0.020	-1.474	0.141	
Propionate	0.008	0.013	0.600	0.549	

1129


A
Day 1
Day 2
T0
T1
T2
T3
T4
T5
T6
-57
0
23
40
59
84
time (min)
B
Cortisol (nmol/L)
4.0
3.0
2.0
*
*
*
*
T0
T1
T2
T3
T4
T5
T6
C
Subjective stress ratings
4.0
3.5
3.0
2.5
Stress group
Control group
*
T0
T1
T2
T3
T4
T5
T6


A
Gut microbial alpha diversity
Cortisol stress reactivity
p = 0.003
0.06
0.04
0.02
0.00
-0.02
-0.04
-3 -2 -1 0 1
Shannon Index
p < 0.001
0.00
-0.02
-0.04
-0.06
Female
Male
Sex
B
Cortisol stress reactivity
p = 0.001
0.04
0.02
0.00
−0.02
−0.04
−0.06
−2 −1 0 1 2
Inverse Simpson
C
p = 0.03
0.04
0.02
0.00
−0.02
−0.04
−0.06
−0.08
−2 −1 0 1 2
Observed richness
D
Post-stress recovery
p = 0.057
0.00
−0.01
−0.02
−0.03
−0.04
−2 −1 0 1 2
Observed richness
p = 0.01
−0.010
−0.015
−0.020
−0.025
−0.030
−0.035
−0.040
Female
Male
Sex

Gut microbial SCFA production capacity