**Effects of a multi-strain probiotic on hippocampal structure and function, cognition, and emotional well-being in healthy individuals: A double blind randomized-controlled trial**

# Supplementary Material

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## 1.1 Additional behavioral assessments

All self-reports were assessed referring to the last two weeks and assessed with validated German versions. Additional indicators of psychological symptoms were: chronic assessed with the Trier Inventory for Chronic Stress (1), sleepiness assessed with the Epworth Sleepiness Scale (2,3), nervousness, euthymia, dysthymia, and worry with the State Trait Anxiety Depression Inventory (4), emotion regulation (functional vs. dysfunctional) with the Cognitive Emotion Regulation Questionnaire (5,6) and Emotion Regulation Questionnaire (ERQ; assessing reappraisal vs. suppression) (7,8), maladaptive looming style (i.e., mental magnification of potential threats and dangers – both social and physical) with the Looming Maladaptive Style Questionnaire (9), empathy with the Reading the Mind in the Eyes test – revised version (10,11), self-reported spatial orientation and navigational ability with the Santa Barbara Sense of Direction questionnaire – Freiburg version (12,13), and executive function processing speed with the digit symbol task from the Berlin Intelligence Scale (14).

## 1.2 Additional behavioral analyses and results

To test whether the probiotic supplement vs. placebo differentially affected the aforementioned, additional behavioral outcomes, a series of classical repeated measured analyses were run with SPSS 25 (IBM Corp.), testing for group x time interaction effects. In addition, we computed the Bayes factor for the H0 (BF01) of the interaction effect in JASP (version 0.14.1) to assess evidence in favor of a null effect, in JASP (version 0.14.1.0). BF01> 1 indicate that, relative to H1, H0 more likely applies to the data, BF01< 1 indicate the opposite. Values between 0.5 and 1.5 can be regarded as inconclusive, BF01 ≥ 3 moderate and ≥ 10 strong evidence for H0 relative to H1 (15). The BF01 for the interaction was computed by dividing BF01 for the main effects plus the interaction (i.e., group + time + group x time) by the BF01 for the main effects (i.e., group + time).

As can be seen in Supplementary Table 1, there were no significant interaction effects. The harmonic mean indicated anecdotal to moderate evidence in favor of the null-hypothesis.

**Supplementary Table 1**

|  |  |  |
| --- | --- | --- |
| **Dependent variables** | **Classical interaction effect statistic** | **BF01** |
| Mental health & sleep variables |
| Chronic stress (TICS) | *F* (1,57) = 1.09, *p* = .300, η2p = .019 | 2.67 |
| ESS–sleepiness | *F* (1,55) = 0.80, *p* = .376, η2p = .014 | 2.69 |
| STADI–nervousness | *F* (1,57) = 0.23, *p* = .632, η2p = .004 | 3.69 |
| STADI-worry | *F* (1,57) = 0.33, *p* = .570, η2p = .006 | 3.25 |
| STADI-euthymia | *F* (1,57) = 0.32, *p* = .573, η2p = .006 | 3.06 |
| STADI-dysthymia | *F* (1,57) = 2.48, *p* = .121, η2p = .042 | 1.39 |
| Emotion regulation, empathy |
| CERQ-dysfunctional ER | *F* (1,53) = 0.28 *p* = .599, η2p = .005 | 3.27 |
| CERQ-functional ER | *F* (1,53) = 0.12 *p* = .726, η2p = .002 | 3.55 |
| ERQ-cognitive reappraisal | *F* (1,54) = 1.40, *p* = .247, η2p = .025 | 2.11 |
| ERQ-suppression | *F* (1,54) = 0.56, *p* = .457, η2p = .010 | 3.15 |
| LMSQ-social looming | *F* (1,54) = 0.55, *p* = .461, η2p = .010 | 3.02 |
| LMSQ-physical looming | *F* (1,55) = 0.79, *p* = .377, η2p = .014 | 2.80 |
| Empathy (reading eyes) | *F* (1,57) = 0.30, *p* = .584, η2p = .005 | 3.29 |
| Cognition |
| SBOD-Sense of direction  | *F* (1,54) = 1.38, *p* = .246, η2p = .025 | 2.11 |
| Digit symbol task | *F* (1,55) = 0.02, *p* = .889, η2p = .000 | 3.18 |
| **Harmonic mean BF01** | **--**  | **2.71** |

*Additional analyses with classical test theory for repeated measures ANOVA interaction and Bayesian null-hypothesis test results concerning effects of the probiotic supplement vs. placebo on different outcomes*

 **Note.** BF01 refers to the Bayes Factor for H0 relative to H1concerning the presence of an interaction effect (i.e., group x time). *ESS* = Epworth Sleepiness Scale; *STADI* = State Trait Anxiety Depression Inventory; *CERQ* = Cognitive Emotion Regulation Questionnaire – dysfunctional and functional Emotion Regulation (*ER*); *ERQ* = Emotion Regulation Questionnaire; *LMSQ* = Looming Maladaptive Style Questionnaire; *Empathy task* = Reading the Mind in the Eyes task – adult version total score. All analyses were conducted by originally assigned groups.

## 2. Descriptive data

**Supplementary Table 2**

*Descriptives for extracted structural HC data*

|  |  |  |
| --- | --- | --- |
|  | **Probiotic**(n = 30) | **Placebo**(n = 29) |
| **Structure/ division** | **Pre-test** | **Post-test** | **Pre-test** | **Post-test** |
|  | M (*SD*) | M (*SD*) | M (*SD*) | M (*SD*) |
| Left HC FreeSurfer7 | 4140.45 (458.71) | 4124.43 (465.39) | 4204.45 (572.19) | 4209.62 (592.79) |
| Right HC FreeSurfer7 | 4212.66 (461.06) | 4186.10 (468.02) | 4467.25 (394.13) | 4484.17 (400.98) |
| CA1 (Cornu Ammonis subfield 1) | 2768.72 (203.65) | 2776.46 (234.99) | 2775.88 (600.18) | 2847.97 (305.23) |
| CA2 (Cornu Ammonis subfield 2) | 51.98 (9.36) | 53.02 (7.94) | 50.96 (15.55) | 53.06 (10.66) |
| CA3 (Cornu Ammonis subfield 3) | 90.72 (25.44) | 90.66 (25.03) | 99.05 (32.15) | 99.82 (26.91) |
| DG (Dentate Gyrus) | 1689.97 (175.27) | 1685.54 (170.87) | 1665.05 (398.46) | 1721.54 (195.55) |
| SUB (Subiculum) | 877.17 (146.64) | 885.41 (124.94) | 855.47 (239.84) | 886.65 (141.03) |
| ERC (Entorhinal Cortex) | 889.14 (151.48) | 890.63 (158.39) | 895.38 (232.51) | 952.52 (137.40) |
| BA35 (perirhinal area 35) | 1168.36 (133.57) | 1174.59 (123.41) | 1188.14 (227.78) | 1221.74 (179.24) |
| BA36 (ectorhinal area 36) | 4215.52 (379.97) | 4196.24 (399.09) | 4194.25 (740.41) | 4309.31 (495.52) |
| PHC (parahippocampal cortex) | 2117.85 (318.01) | 2117.96 (342.07) | 2128.83 (397.76) | 2149.87 (353.32) |
| HC sulcus (hippocampal sulcus) | 646.95 (166.29) | 663.52 (181.73) | 707.28 (169.60) | 676.41 (174.44) |

**Note.** All analyses were conducted by originally assigned groups.

**Supplementary Table 3**

*Descriptive data for cognitive tests and self-reports on psychiatric symptoms, stress, and emotion regulation reported in main paper*

|  |  |  |
| --- | --- | --- |
|  | **Probiotic**(n = 30) | **Placebo**(n = 29) |
|  | Pre-test | Post-test | Pre-test | Post-test |
| **Tests/ self-reports** | M (*SD*) | M (*SD*) | M (*SD*) | M (*SD*) |
|  | spatial memory and navigation |
| Corsi block tapping – performance score | 55.00 (19.06) | 58.43 (21.57) | 62.43 (19.42) | 65.96 (18.03) |
| BIS-4 Map - total score | 14.39 (5.45) | 18.46 (5.27) | 14.48 (4.87) | 19.48 (8.40) |
| OLMT - total score | 14.25 (4.52) | 14.11 (4.92) | 16.52 (3.81) | 17.72 (3.46) |
| Tunnel task allocentric ratio (%) | 36.46 (42.82) | 19.58 (15.81) | 29.16 (31.17) | 18.09 (20.02) |
| Tunnel task mean deviation (angle) | 50.83 (39.22) | 61.67 (43.10) | 45.60 (35.25) | 45.20 (39.70) |
|  | Verbal memory |
| RBMT - immediate recall total | 10.48 (3.59) | 13.03 (3.49) | 11.43 (3.54) | 14.30 (3.51) |
| RBMT - delayed recall total | 9.51 (3.38) | 12.40 (3.64) | 10.50 (3.67) | 13.14 (3.63) |
|  | Mental health & emotion regulation |
| BDI-II | 5.57 (6.25) | 3.80 (4.72) | 4.97 (5.95) | 4.83 (5.56) |
| Total psychopathology (GSI) | 12.37 (11.68) | 11.57 (9.40) | 13.68 (11.46) | 12.43 (11.08) |
| BSI depression  | 1.20 (1.47) | 1.13 (1.57) | 1.48 (2.21) | 1.31 (1.85) |
| BSI anxiety | 1.27 (1.44) | 1.37 (1.69) | 1.76 (1.48) | 1.21 (1.40) |
| PSS | 21.07 (4.93) | 21.10 (6.01) | 22.10 (6.62) | 22.41 (6.28) |
| RSQ - symptom-related rumination | 12.14 (4.12) | 11.83 (2.73) | 12.32 (3.56) | 12.68 (3.36) |
| RSQ - self-related rumination | 13.41 (4.57) | 13.31 (3.53) | 14.61 (3.57) | 14.14 (3.73) |
| RSQ - distraction | 20.28 (4.08) | 20.34 (4.96) | 20.32 (3.51) | 20.25 (4.36) |

**Note.** *Corsi* = Corsi block tapping task; *BIS-4 Map* = map subtest from Berlin Intelligence Scale 4, total score (correctly recalled route segments), *OLMT* = Object Location Memory Task (no. of correctly placed objects both in sequence and position); *RBMT* = Rivermead Behavioral Memory Test, *BDI-II* = Beck’s Depression Inventory-II revised; *GSI* = Global Severity Index (Brief Symptom Inventory); *BSI* depression = Brief Symptom Inventory depression subscale; BSI anxiety = Brief Symptom Inventory anxiety subscale; *RSQ* = Response Style Questionnaire (subscales). All analyses were conducted by originally assigned groups.

|  |  |  |
| --- | --- | --- |
|  | **Probiotic**(n = 30) | **Placebo**(n = 29) |
|  | Pre-test | Post-test | Pre-test | Post-test |
| **Tests/ self-reports** | M (*SD*) | M (*SD*) | M (*SD*) | M (*SD*) |
|  | Sleep & mental health |
| ESS–sleepiness | 8.00 (4.24) | 8.63 (4.14) | 7.56 (3.61) | 8.70 (3.61) |
| STADI–nervousness | 6.87 (2.06) | 6.90 (2.06) | 6.55 (1.66) | 6.90 (1.86) |
| STADI-worry | 9.07 (2.77) | 8.53 (2.78) | 9.21 (3.91) | 9.24 (3.08) |
| STADI-euthymia | 10.90 (3.07) | 10.83 (3.33) | 11.03 (2.83) | 11.38 (3.31) |
|  | Emotion regulation & empathy |
| CERQ-dysfunctional ER | 24.29 (5.03) | 22.93 (4.85) | 25.56 (5.62) | 25.00 (4.50) |
| CERQ-functional ER | 55.96 (8.48) | 55.61 (10.19) | 51.56 (11.58) | 52.00 (11.54) |
| ERQ-cognitive reappraisal | 30.24 (5.26) | 32.03 (4.58) | 29.93 (5.34) | 30.37 (5.31) |
| ERQ-suppression | 13.93 (4.84) | 14.59 (4.87) | 14.15 (5.34) | 15.59 (6.23) |
| LMSQ-social looming | 35.79 (8.76) | 35.28 (7.42) | 37.44 (6.33) | 38.07 (7.86) |
| LMSQ-physical looming | 37.41 (8.46) | 36.83 (8.29) | 40.93 (8.07) | 41.96 (7.26) |
| Empathy task | 25.73 (3.46) | 26.07 (4.20) | 26.97 (2.86) | 27.83 (2.61) |

**Supplementary Table 4**

*Descriptive data on additional outcomes comprising self-reports on sleepiness, psychiatric symptoms, stress, emotion regulation, and empathy*

**Note.** *ESS* = Epworth Sleepiness Scale; *STADI* = State Trait Anxiety Depression Inventory; *CERQ* = Cognitive Emotion Regulation Questionnaire – dysfunctional and functional Emotion Regulation (*ER*); *ERQ* = Emotion Regulation Questionnaire; *LMSQ* = Looming Maladaptive Style Questionnaire; *Empathy task* = Reading the Mind in the Eyes task – adult version total score. All analyses were conducted by originally assigned groups.

## 3. Resting state fMRI preprocessing

For the functional images from the resting state sequence, to ensure steady-state longitudinal magnetization, the first 10 images were discarded. Slice timing was performed, and the data realigned. Individual T1 images were co-registered to functional images. Afterwards segmentation into grey matter, white matter and cerebrospinal fluid was conducted. Data was spatially normalized to the MNI template and then spatially smoothed with 6-mm FWHM, to improve signal-to-noise ratio. Motion and signals from white matter and cerebrospinal fluid were regressed, followed by data filtering (0.01 – 0.09 Hz) to reduce physiological high-frequency respiratory and cardiac noise and low-frequency drift. Finally, the data was detrended. All steps of data preprocessing were done using, SPM12 except filtering that was applied using REST toolbox. SPM12 and REST toolbox ran under MATLAB 2017a ([www.mathworks.com](http://www.mathworks.com)).

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# Nutritional information for placebo milk powder

Based on nutritional data for 13.0g Bebivita (dissolved in 100 ml water), provided on packaging by the manufacturer. Thus, the data was divided by 2.95 (thus approximating nutritional values for a dose of 4.4g, as taken in daily by the participants of the placebo group, once per day for four weeks).

**Supplementary Table 5**

*Nutritional information for the placebo preparation*

|  |
| --- |
| **Nutritional data for 4.4 g Infant Formula Milk Bebivita® Pre, approximated values based on values provided by the manufacturer** |
| Energy | 95 kj/ 23 kcal |
| Fat, total  | 1.20 g |
|  staurated fats | 0.41 g |
|  monounsaturated fatty acids | 0.58 g |
|  polyunsaturated fatty acids | 0.24 g |
| Carbohydrates, total | 2.51 g |
|  Sugar (lactose) |  2.51 g |
| Dietary fibers | < 0.03 g |
| Protein | 0.47 g |
| Salt | 16.95 mg |
| Sodium | 6.78 mg |
| Potassium | 25.42 mg |
| Chloride | 14.24 mg |
| Calcium | 16.95 mg |
| Phosphorus | 9.49 mg |
| Magnesium | 2.03 mg |
| Iron | 0.17 mg |
| Zinc | 0.20 mg |
| Copper | 0.01 mg |
| Iodine | 5.08 µg |
| Selene | 0.54 µg |
| Manganese | 0.002 mg |
| Fluoride | 0.004 mg |
| Vitamine A | 23.73 µg |
| Vitamine D | 0.34 µg |
| Vitamine E | 0.28 mg |
| Vitamine K | 1.69 µg |
| Vitamine C | 3.73 mg |
| Vitamine B1 | 0.024 mg |
| Vitamine B2 | 0.034 mg |
| Niacin | 0.153 mg |
| Vitamin B6 | 0.0203 mg |
| Folic acid | 4.07 µg |
| Vitamine B12 | 0.068 µg |
| Pantothenic acid | 0.169 mg |
| Biotin | 0.576 µg |
| Choline | 4.07 mg |
| Inosit | 2.0 mg |
| Linoleum acid (Omega 6) | 0.203 g |
| Linoleum acid (Omega 3) | 0.024 g |