

SUPPLEMENTARY INFORMATION

Probiotic effects on brain health are both transient and sustained for several weeks after discontinuation: a randomised controlled trial

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Supplementary Information 1. Sensitivity analysis.

To evaluate robustness of the two-way ANOVA analyses of psychological symptom ratings (HADS Total Score, HADS Anxiety Subscore, HADS Depression Subscore, PSS, PSQI), non-parametric Friedman tests were assessed per group (encapsulated probiotic, non-encapsulated probiotic, placebo) with *posthoc* Dunn's multiple comparisons comparing discontinuation follow-up with every other timepoint, and non-parametric Kruskal-Wallis tests were performed between groups with *posthoc* Dunn's multiple comparisons at discontinuation follow-up (week 10-12). Friedman, Kruskal-Wallis, and Dunn's test results are presented if $p < 0.1$.

HADS Total Score within the encapsulated probiotic group between timepoints differed near-significantly (Friedman statistic=7.500, $p=0.0576$), *posthoc* Dunn's multiple comparison test revealed that this difference originated from a decrease from intervention end to discontinuation follow-up ($p=0.0118$, adjusted $p=0.0355$).

HADS Anxiety Subscore within the encapsulated probiotic group between timepoints differed near-significantly (Friedman statistics=7.456, $p=0.0587$), *posthoc* Dunn's multiple comparison tests revealed that this difference originated from a decrease from intervention end to discontinuation follow-up ($p=0.0201$, adjusted $p=0.0604$).

HADS Anxiety Subscore within the non-encapsulated probiotic group between timepoints differed near-significantly (Friedman statistics=7.780, $p=0.0508$), *posthoc* Dunn's multiple comparison tests were not significant.

No other within-group (i.e. between-timepoint) differences were observed for any other group or score. Neither were any between-group differences observed at discontinuation follow-up.

Those results are consistent with the results observed by two-way ANOVA and *posthoc* Holm-Sidak's multiple comparisons as reported in the main text and Figure 4, supporting their robustness.

Supplementary Table 1. Psychological symptom ratings compared between all timepoint and intervention groups in this sub-population (n=32), no multiplicity correction applied.

	HADS total			HADS anxiety			HADS depression			PSS			PSQI		
	Mean diff.	95.00% CI of diff.	p-value	Mean diff.	95.00% CI of diff.	p-value	Mean diff.	95.00% CI of diff.	p-value	Mean diff.	95.00% CI of diff.	p-value	Mean diff.	95.00% CI of diff.	p-value
Baseline															
Encapsulated probiotic vs Non-encapsulated probiotic	3.42	-3.03 to 9.87	0.2583	2.71	-1.56 to 6.98	0.1827	0.71	-1.88 to 3.30	0.5517	3.54	-1.62 to 8.70	0.1587	1.83	-1.57 to 5.23	0.2601
Encapsulated probiotic vs Placebo	3.58	-2.96 to 10.13	0.2488	2.96	-1.34 to 7.26	0.1535	0.63	-2.02 to 3.27	0.6118	2.63	-2.64 to 7.89	0.2988	0.11	-3.54 to 3.75	0.9504
Non-encapsulated probiotic vs Placebo	0.17	-2.75 to 3.08	0.9063	0.25	-1.50 to 2.00	0.7694	-0.08	-1.50 to 1.33	0.9036	-0.92	-4.34 to 2.50	0.5835	-1.72	-4.25 to 0.81	0.1710
Week 3															
Encapsulated probiotic vs Non-encapsulated probiotic	4.00	-3.43 to 11.43	0.2493	3.33	-1.34 to 8.00	0.1390	0.67	-2.40 to 3.73	0.6313	3.04	-0.67 to 6.75	0.1001	1.49	-1.69 to 4.68	0.3332
Encapsulated probiotic vs Placebo	5.67	-1.81 to 13.14	0.1195	4.33	-0.34 to 9.01	0.0652	1.33	-1.74 to 4.41	0.3502	2.13	-1.79 to 6.04	0.2658	0.61	-2.66 to 3.87	0.6978
Non-encapsulated probiotic vs Placebo	1.67	-0.99 to 4.32	0.2054	1.00	-0.71 to 2.71	0.2383	0.67	-0.56 to 1.89	0.2702	-0.92	-4.14 to 2.30	0.5603	-0.89	-3.70 to 1.93	0.5205
Week 6															
Encapsulated probiotic vs Non-encapsulated probiotic	5.71	-1.64 to 13.05	0.1108	4.71	0.21 to 9.21	0.0424	1.00	-2.52 to 4.52	0.5323	3.13	-1.85 to 8.10	0.1921	2.04	-1.08 to 5.15	0.1789
Encapsulated probiotic vs Placebo	5.63	-1.87 to 13.12	0.1246	4.21	-0.36 to 8.77	0.0667	1.42	-2.16 to 4.99	0.3955	3.63	-1.50 to 8.75	0.1490	0.94	-2.45 to 4.33	0.5624
Non-encapsulated probiotic vs Placebo	-0.08	-3.28 to 3.11	0.9569	-0.50	-2.28 to 1.28	0.5642	0.42	-1.12 to 1.96	0.5787	0.50	-2.62 to 3.62	0.7420	-1.10	-3.64 to 1.44	0.3793
Week 10-12															
Encapsulated probiotic vs Non-encapsulated probiotic	2.79	-3.75 to 9.33	0.3592	2.63	-1.73 to 6.98	0.2058	0.17	-2.64 to 2.97	0.8969	3.13	-1.91 to 8.16	0.2040	2.58	-0.512 to 5.67	0.0945
Encapsulated probiotic vs Placebo	3.29	-3.57 to 10.15	0.3156	2.88	-1.57 to 7.32	0.1808	0.42	-2.60 to 3.44	0.7708	2.42	-2.59 to 7.42	0.3178	1.40	-1.92 to 4.72	0.3834
Non-encapsulated probiotic vs Placebo	0.50	-3.44 to 4.44	0.7930	0.25	-2.05 to 2.55	0.8233	0.25	-1.73 to 2.23	0.7940	-0.71	-4.75 to 3.34	0.7198	-1.18	-3.80 to 1.44	0.3600
Encapsulated probiotic															
Baseline vs Week 3	-0.75	-2.28 to 0.78	0.2849	-0.63	-1.62 to 0.37	0.1803	-0.13	-0.95 to 0.70	0.7318	2.00	-1.13 to 5.13	0.1743	-0.50	-1.60 to 0.59	0.3159
Baseline vs Week 6	-1.63	-3.03 to -0.22	0.0294	-1.00	-2.10 to 0.09	0.0676	-0.63	-1.62 to 0.37	0.1803	1.25	-1.81 to 4.31	0.3656	-0.63	-2.46 to 1.21	0.4481

Baseline vs Week 10-12	0.38	-0.39 to 1.14	0.2849	0.25	-0.49 to 0.99	0.4512	0.13	-0.41 to 0.66	0.5983	1.50	0.02 to 2.98	0.0479	-0.75	-2.07 to 0.57	0.2216
Week 3 vs Week 6	-0.88	-2.39 to 0.64	0.2133	-0.38	-1.26 to 0.51	0.3506	-0.50	-1.60 to 0.59	0.3159	-0.75	-4.06 to 2.56	0.6083	-0.13	-1.34 to 1.09	0.8153
Week 3 vs Week 10-12	1.13	-0.57 to 2.82	0.1612	0.88	-0.07 to 1.82	0.0639	0.25	-0.91 to 1.41	0.6263	-0.50	-3.22 to 2.22	0.6767	-0.25	-1.90 to 1.40	0.7318
Week 6 vs Week 10-12	2.00	0.66 to 3.34	0.0096	1.25	0.18 to 2.32	0.0282	0.75	-0.12 to 1.62	0.0796	0.25	-2.93 to 3.43	0.8580	-0.13	-2.42 to 2.17	0.9013
Non-encapsulated probiotic															
Baseline vs Week 3	-0.17	-1.10 to 0.77	0.7014	0.00	-0.47 to 0.47	>0.9999	-0.17	-0.97 to 0.64	0.6576	1.50	-0.27 to 3.27	0.0884	-0.83	-1.80 to 0.14	0.0854
Baseline vs Week 6	0.67	-0.28 to 1.62	0.1513	1.00	-0.19 to 1.81	0.0204	-0.33	-0.83 to 0.16	0.1661	0.83	-0.72 to 2.39	0.2623	-0.42	-1.21 to 0.37	0.2691
Baseline vs Week 10-12	-0.25	-1.81 to 1.31	0.7308	0.17	-1.10 to 1.43	0.7774	-0.42	-1.29 to 0.46	0.3177	1.08	-0.72 to 2.89	0.2137	0.00	-0.47 to 0.47	>0.9999
Week 3 vs Week 6	0.83	-0.31 to 1.98	0.1372	1.00	0.19 to 1.81	0.0204	-0.17	-0.87 to 0.54	0.6147	-0.67	-2.32 to 0.99	0.3944	0.42	-0.46 to 1.29	0.3177
Week 3 vs Week 10-12	-0.08	-1.45 to 1.28	0.8957	0.17	-1.13 to 1.46	0.7822	-0.25	-1.07 to 0.57	0.5152	-0.42	-2.36 to 1.53	0.6462	0.83	-0.17 to 1.84	0.0960
Week 6 vs Week 10-12	-0.92	-2.05 to 0.22	0.1023	-0.83	-1.77 to 0.10	0.0748	-0.08	-0.77 to 0.61	0.7949	0.25	-1.49 to 1.99	0.7574	0.42	-0.42 to 1.25	0.2945
Placebo															
Baseline vs Week 3	1.33	-0.003 to 2.67	0.0504	0.75	-0.07 to 1.57	0.0688	0.58	-0.37 to 1.54	0.2064	1.50	-0.68 to 3.68	0.1574	0.00	-0.78 to 0.78	>0.9999
Baseline vs Week 6	0.42	-1.22 to 2.05	0.5863	0.25	-0.87 to 1.37	0.6332	0.17	-0.59 to 0.92	0.6380	2.25	-0.19 to 4.69	0.0673	0.21	-1.01 to 1.43	0.7148
Baseline vs Week 10-12	0.08	-2.35 to 2.51	0.9412	0.17	-1.36 to 1.70	0.8148	-0.08	-1.25 to 1.08	0.8776	1.29	-1.63 to 4.21	0.3511	0.54	-0.82 to 1.91	0.4009
Week 3 vs Week 6	-0.92	-2.44 to 0.60	0.2112	-0.50	-1.42 to 0.42	0.2562	-0.42	-1.21 to 0.37	0.2691	0.75	-1.19 to 2.69	0.4123	0.21	-0.50 to 0.92	0.5315
Week 3 vs Week 10-12	-1.25	-3.69 to 1.19	0.2835	-0.58	-1.65 to 0.48	0.2534	-0.67	-2.18 to 0.85	0.3541	-0.21	-3.01 to 2.59	0.8729	0.54	-0.35 to 1.44	0.2088
Week 6 vs Week 10-12	-0.33	-1.94 to 1.28	0.6576	-0.08	-1.08 to 0.91	0.8569	-0.25	-1.19 to 0.69	0.5715	-0.96	-2.72 to 0.80	0.2555	0.33	-0.58 to 1.25	0.4382

Supplementary Table 2. Resting state functional connectivity at discontinuation follow-up (week 10-12) versus baseline. Significant time*group interactions; all voxel threshold $p < 0.001$ uncorrected and cluster threshold $p < 0.05$ with exact p -value as indicated (hypothesis-driven seed-to-voxel analysis (two-sided), regions annotation based on Harvard-Oxford atlas). Table only reports significant alterations, yet all 21 a priori defined regions of interest were tested as seeds. None of those effects remained significant, when additional multiplicity correction is applied for the number of seeds tested, comparisons of two pairs of timepoints and of three intervention groups (cluster size $p < 0.0004$ FDR-corrected).

Group comparison	A priori selected seed region	Target cluster size [number of voxels]	Target cluster-size p-FDR corrected	Target cluster peak coordinate [xx yy zz]	Target cluster coverage (order of anatomical regions based on proportion of coverage from largest to smallest)	Intervention-related alterations of connectivity [T]	Intervention-related alterations of connectivity ^{##}
Placebo vs Encapsulated probiotic	Superior Frontal gyrus Left [#]	135	0.0455	-48 +4 -18	Temporal Pole Left, Superior	-6.66	- anti-correlation
					Temporal Gyrus anterior		changed to
					division Left, Middle		correlation upon
					Temporal Gyrus anterior		intake and
					division Left, Planum Polare		subsequent
				Left			discontinuation of
							the encapsulated
							probiotic
							- correlation alteration
							was neglectable
							upon intake and
							subsequent

							discontinuation of the placebo
							- anti-correlation decreased upon intake and subsequent discontinuation of the non-encapsulated probiotic
Placebo vs Non-encapsulated probiotic	Hippocampus Left [#]	233	0.0026	-44 +38 +6	Frontal Pole Left, Inferior Frontal Gyrus pars triangularis Left [#]	+6.17	- correlation decreased upon intake and subsequent discontinuation of the placebo
		105	0.0432	+36 -84 +26	Lateral Occipital Cortex superior division Right	-6.02	- anti-correlation increased upon intake and subsequent discontinuation of

						the non-encapsulated probiotic
						- anti-correlation
						changed to
						correlation upon
						intake and
						subsequent
						discontinuation of
						the placebo
						- anti-correlation
						decreased upon
						intake and
						subsequent
						discontinuation of
Amygdala Left [#]	197	0.0059	-38 +44 -4	Frontal Pole Left, Inferior Frontal Gyrus pars triangularis Left [#] , Frontal Orbital Cortex Left	+5.75	the non-encapsulated probiotic
						- correlation
						decreased upon
						intake and
						subsequent

							discontinuation of the placebo
							- anti-correlation increased upon intake and subsequent discontinuation of the non-encapsulated probiotic
Encapsulated probiotic vs Non-encapsulated probiotic	Middle Frontal Gyrus Right#	189	0.0141	-24 -32 -28	Cerebellum Left, Brainstem, Parahippocampal Gyrus posterior division Left, Temporal Fusiform Cortex posterior division Left	+10.25	- anti-correlation changed to correlation upon intake and subsequent discontinuation of the encapsulated probiotic

#*a priori* defined region of interest.

##Synchronised brain activity patterns are described as correlations (e.g. two brain regions showing the same activity pattern) and anti-correlations (e.g. two brain regions showing opposite activity patterns), direction of correlation was interpreted as changed (from correlation to anti-correlation or vice versa), when error bar (based on standard error of the mean across individual voxels) did not cross zero

Supplementary Table 3. Resting state functional connectivity at discontinuation follow-up (week 10-12) versus intervention end (week 6). Significant time*group interactions; all voxel threshold $p < 0.001$ uncorrected and cluster threshold $p < 0.05$ with exact p -value as indicated (hypothesis-driven seed-to-voxel analysis (two-sided), regions annotation based on Harvard-Oxford atlas). Table only reports significant alterations, yet all 21 a priori defined regions of interest were tested as seeds. In italics highlighted alterations remain significant, when additional multiplicity correction is applied for the number of seeds tested, comparisons of two pairs of timepoints and of three intervention groups (cluster size $p < 0.0004$ FDR-corrected).

Group comparison	<i>A priori</i> selected seed region	Target cluster size [number of voxels]	Target cluster-size p-FDR corrected	Target cluster peak coordinate [xx yy zz]	Target cluster coverage (order of anatomical regions based on proportion of coverage from largest to smallest)	Intervention-related alterations of connectivity [T]	Intervention-related alterations of connectivity ^{##} [description]
Placebo vs Encapsulated probiotic	---	none	---				
Placebo vs Non-encapsulated probiotic	Middle Frontal Gyrus Right [#]	159	0.0298	+6 -76 -12	Lingual Gyrus Right, Vermis 6, Cerebellum Right	-7.22	- correlation changed to anti-correlation upon discontinuation of the non-encapsulated probiotic - anti-correlation changed to correlation upon

							discontinuation of the placebo
							- correlation increased upon discontinuation of the non-encapsulated probiotic
	Inferior Frontal Gyrus pars opercularis Left [#]	253	0.0016	+2 -82 +10	Supracalcarine Cortex Right & Left, Occipital Pole Right & Left, Intracalcarine Cortex Right & Left, Cuneal Cortex Right & Left	+5.18	- correlation changed to anti-correlation upon discontinuation of the placebo
							- correlation decreased upon discontinuation of the non-encapsulated probiotic
	Precuneus [#]	148	0.0182	-2 -44 +30	Cingulate Gyrus posterior division [#]	-7.04	- correlation increased upon discontinuation of the placebo
Encapsulated probiotic vs	Superior Parietal Lobule Left [#]	379	0.0001	+20 -96 +30	Occipital Pole Right, Lateral Occipital Cortex superior division Right, Cuneal Cortex Right, Intracalcarine Cortex Right, Supracalcarine Cortex Right	+7.38	- correlation decreased upon discontinuation of the non-encapsulated probiotic

Non-encapsulated probiotic							- correlation increased upon discontinuation of the encapsulated probiotic
							- correlation increased upon discontinuation of the non-encapsulated probiotic
Supramarginal Gyrus posterior division Right [#]	145	0.0270	+60 -22 +36	Supramarginal Gyrus anterior division Right [#] , Postcentral Gyrus Right	-6.41		- correlation decreased upon discontinuation of the encapsulated probiotic

[#]*a priori* defined region of interest.

^{##}Synchronised brain activity patterns are described as correlations (e.g. two brain regions showing the same activity pattern) and anti-correlations (e.g. two brain regions showing opposite activity patterns), direction of correlation was interpreted as changed (from correlation to anti-correlation or vice versa), when error bar (based on standard error of the mean across individual voxels) did not cross zero