Study title: Acute and Chronic Effects of Ecologic Barrier© on Mood and Cognition

Acronym: ProCog

Date: 29/12/2020

ProCog Study Design & Protocol

Aims

To investigate the acute (24 hours) and chronic (4/8 weeks) effects of probiotics (Ecologic Barrier) on cognitive performance and mood in ageing adults.

Design

Double-blind, cross-over, randomised control trial. All outcomes measured at baseline, 23 hours, 4 weeks and 8 weeks (*see page 3 for explanation*).

Intervention

Ecologic Barrier© – daily intervention of 2g (5 billion CFU per day). Placebo (carrier) matched in appearance, smell and taste.

Cognitive Outcomes

Based on the literature review to-date, exact tasks to be confirmed (*see page 10 for justifications*). Parallel versions required for multiple test points.

- An emotional recognition/decision task
- Rey Auditory Verbal Learning Test (RAVLT)
- Corsi blocks
- Switching task

Mood Outcomes

Based on the literature review to-date, exact tasks to be confirmed (*see page 10 for justifications*).

- Leiden Index of Depression Sensitivity revised (LEIDS-r)
- Positive and Negative Affect Schedule expanded (PANAS-x)
- State items from State Trait Anxiety Inventory

Additional outcomes/possible covariates *(see page 6 for justifications)*

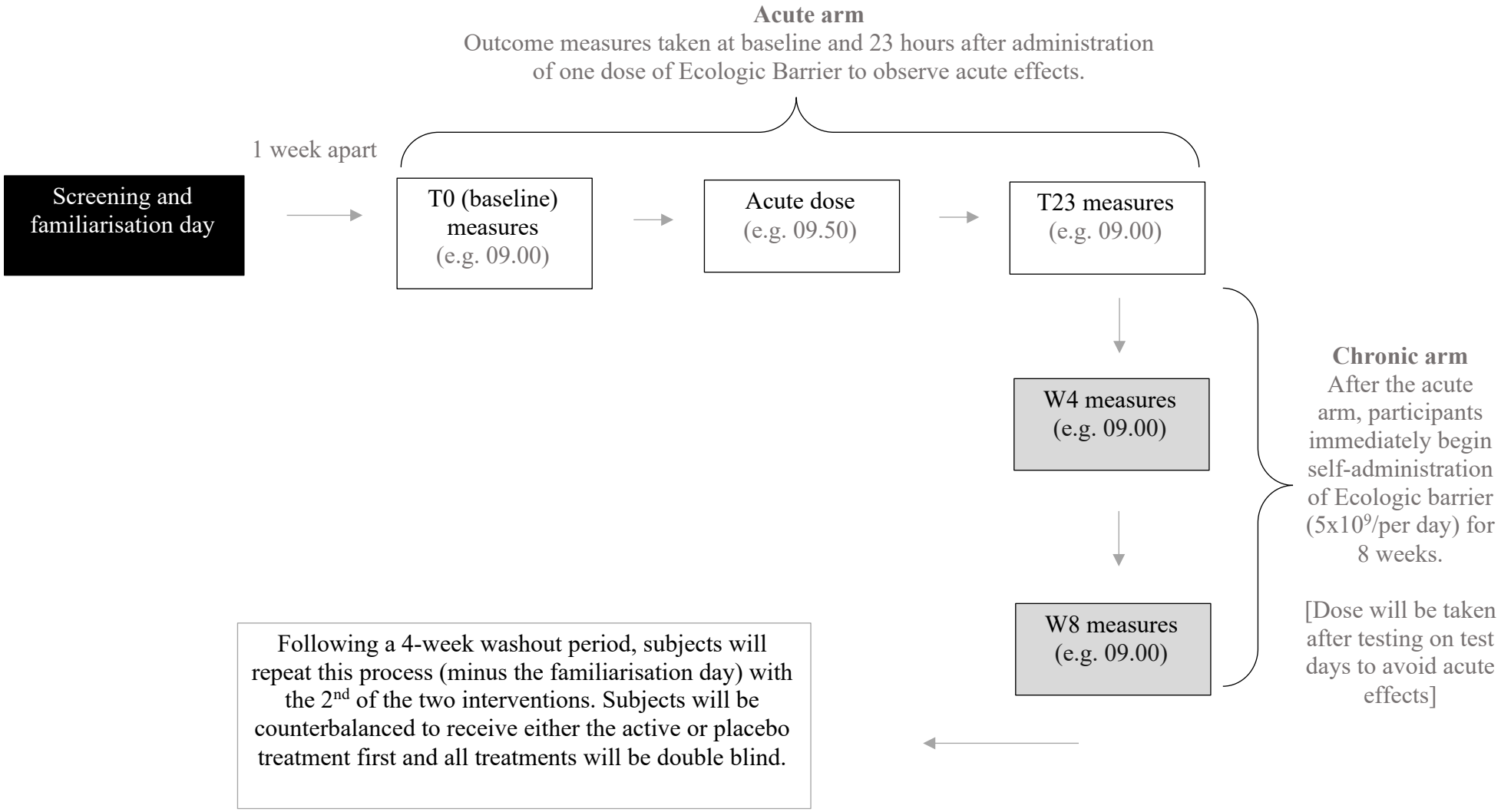
- Dietary measures with a food frequency questionnaire (FFQ)
- Two-day food diary to be completed over the acute arm
- Perceived Stress Scale
- Centre for Epidemiological Studies Depression Scale (CESD)
- Faecal microbiota profile (baseline and 8 weeks. Samples will also be collected at 4 weeks with analysis subject to cognitive results)

No. of Participants: 30 based on previous studies and G*Power calculation, see page 11.

Exclusion criteria:

Already a habitual user of probiotics, antibiotic treatment within last 3 months, no current diagnosis of and not currently receiving treatment for mental health disorder, gastrointestinal disorder, allergic to any ingredient of active or placebo treatment. Regular smoker. Participants must not consume probiotic or prebiotics supplements or live yoghurt throughout the course of study.

Schematic diagram of cross-over RCT



Upon initial enquiry
- A list of inclusion/exclusion criteria will be provided for participant to self-assess eligibility.
Familiarisation day
- Double check that participants meet inclusion/ exclusion criteria - Take demographic information (see below) - Minimum 2 practice sessions of battery (one guided and one unsupervised) - Provide first sample kit with instructions

T0 (baseline)
- Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale - Provide 2-day food diary to complete
[administer intervention immediately after baseline battery]
T24
- Standardised breakfast - Cognitive/mood battery - Supply with 4 weeks' worth of treatment A and administration instructions - Provide FFQ to complete

W4
- Compliance check - Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale - Supply further 4 weeks' worth of treatment A
W8
- Compliance check - Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale

T0 (baseline arm 2)
- Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale - Provide 2-day food diary to complete
[administer intervention immediately after baseline battery]
T23 (arm 2)
- Standardised breakfast - Cognitive/mood battery - Supply with 4 weeks' worth of treatment B and administration instructions

W4 (arm 2)
- Compliance check - Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale - Supply further 4 weeks' worth of treatment B
W8 (arm 2)
- Compliance check - Collect faecal sample - Standardised breakfast - Cognitive/mood battery - Perceived Stress Scale

Demographic information to be collected

Age

Gender

Ethnicity

Education level

Socio Economic Status

Height/weight/BMI

Regular medications

Smoking status – smoking can affect both the gut microbiome[1] and cognitive function, therefore useful to know.

Alcohol consumption – as above[2], although perhaps only an effect of chronic drinking rather than acute or low/moderate alcohol consumption[3] → measured using AUDIT

> Alcohol Use Disorders Identification Test (AUDIT) – short 10 item questionnaire developed by the WHO to assess alcohol consumption and highlight potential alcohol problems. https://www.drugabuse.gov/sites/default/files/files/AUDIT.pdf

Additional measures

Perceived stress – previous studies suggest that probiotic intervention is effective at protecting cognitive function under acute stress[4,5,6]. Unclear whether this would apply to general perceived stress as opposed to physically induced stress. Use Perceived Stress Scale (PSS) to measure perceived stress and potentially include as a covariate. Some studies also used the PSS as an outcome measure to see if a probiotic intervention itself has an influence on perceived stress. This outcome will be measured at baseline and all chronic timepoints.

> Perceived Stress Scale – well validated measure of perceived stress drawing on thoughts and feelings from the past month. Has also been used previously as an outcome measure to examine whether a probiotic intervention can influence perceived stress, with mixed findings[7,8].https://www.northottawawellnessfoundation.org/wp-content/uploads/2018/04/PerceivedStressScale.pdf.

A measure of habitual diet – useful to know the habitual diet of the participants. Also, this may enable us to explore the idea that individual microbiome composition will influence response to probiotic intervention. Those with a naturally more diverse gut microbiota (and therefore possibly more diverse diet) may respond better to the treatment. Or vice versa?[9,10,11,27]

There is therefore a trade-off between wanting to remove diet as a confound when looking at cognitive data and allowing participants to continue eating as normal in order to maintain normal gut composition. Therefore, our decision is to provide the standardised breakfast at

each test point to avoid acute effects of breakfast variation on cognitive performance. Food consumption throughout the rest of the study period won't be controlled but should be recorded in a **2-day food diary during the acute phase**, the rationale here being that it enables participants to maintain their normal diet.

Epic Norfolk FFQ – will be used to gain an idea of habitual diet over last year.

We may also want to include some questions after administration about tolerability of the intervention and any potential side effects.

Example test day timelines (*based on testing no more than two participants at any one time to comply with COVID-19 rules*)

Familiarisation day

09:00
- Check inclusion/ exclusion criteria
- Consent
- Complete all demographic info

09:45
- Complete first assisted cognitive/mood battery

10:45
- Short break if needed

[experimenter to check data]

11:15
- Second run through of battery

12:15
- Confirm happy to continue
- Send home with sample kit and instructions

Baseline

08:30 | 09:15
- Collect faecal sample
- Standardised breakfast

09:00 | 09:45
- Cognitive/mood battery

10:00 | 10:45
- Administer intervention

All other test days (23 hours, 4 and 8 weeks)

08:30 | 09:15
- Collect faecal sample (at 4 & 8 weeks)
- Standardised breakfast

09:00 | 09:45
- Cognitive/mood battery

Using 23 hours (rather than 24) allows us to keep in line with the time at which cognitive baseline measures were recorded. We see significant time of day effects on cognition, so keeping the times consistent within participants at all test points is important to allow more reliable and sensitive assessment of intervention effects

Justification for chosen outcome measures

Outcome measure	Supporting evidence
An emotional recognition/decision task (TBC)	Significant positive effect on both emotional recognition and decision tasks following probiotic intervention compared with placebo [4,12] (both used Winclove multi-strain products) Findings also supported by fMRI data suggesting altered affective processing following supplementation with a fermented milk product[13]
Ray Auditory Verbal Learning Task	Some evidence to support positive effect on verbal memory using AVLTs in a depressed population[14], volunteers with HIV-1[15] (although a few flaws in this study) and healthy adults[16] (although open-label). There is an overlap here of probiotics used in each study, including *Lactobacillus plantarum*, *Bifidobacterium breve* and *Bifidobacterium longum.*
Corsi blocks	Studies have found protective effect of probiotics on WM under stress[4,6,8]. Unclear whether an effect without the acute stressor?
Switching task (from current battery)	A couple of studies used the switching task from CANTAB on children but found no effect, while another reported improved performance in both active and placebo groups in healthy adults[20], probably due to practice effects. One other study suggests a large effect size[16] but is open-label and also likely to practice effects. So unclear from current literature but given potential improvements in attention could be an interesting one to include, as we know this switching task is, to a degree, an endurance test and is sensitive to nutritional interventions.
Leiden Index of Depression Sensitivity revised (LEIDS-r)	Has been used previously in studies supplementing with Ecologic barrier in healthy young adults[21] and patients with MDD[22], where chronic supplementation was associated with a significant reduction in reactivity to sad mood. Would therefore be good to see if these findings persist in an acute trial.
Centre for Epidemiological Studies Depression Scale (CESD)	Well validated measure of depressive symptoms.
Positive and Negative Affect Schedule expanded (PANAS-x)	As there is little evidence regarding how Ecologic barrier may influence general mood, particularly in an acute setup, the PANAS-x

	would allow us to cover 11 affective states (as opposed to the POMS which is longer and covers fewer affective states)
State items from State Trait Anxiety Inventory	Good to include an anxiety measure - chronic supplementation studies have reported reductions in anxiety[23,24]. Beck Anxiety Inventory and General Anxiety Disorder-7 wouldn't work for an acute study as they ask about feelings over the last 2 weeks/month. State items from the STAI therefore work better as they ask about how you feel in that moment.

Generally, these cognitive domains have been chosen as there is evidence from more than one paper using the same or similar tasks for a positive effect of probiotic supplementation on performance. Where possible, have also tried to use tasks that have been associated with improvement following Ecologic barrier or multi-strain interventions containing similar genera.

Sample size power calculation

Previous parallel RCTs looking at the effect of Ecologic Barrier on cognitive or mood outcomes have used between 20 and 36 subjects per group[4, 21, 22, 24]. There are some acute prebiotic studies looking at mood/cognitive outcomes – a positive effect of an intervention was reported using 30 participants in a cross-over RCT[25] and 23 in a parallel groups RCT[26].

Previously, effect sizes between 0.4 and 0.6 have been reported for probiotic intervention on tasks of attention and executive function[28].

G*Power calculation based on a medium effect size of 0.5:

t tests - Means: Difference between two dependent means (matched pairs)

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One
	Effect size dz	=	0.5
	α err prob	=	0.05
	Power (1-β err prob)	=	0.8
Output:	Noncentrality parameter δ	=	2.5980762
	Critical t	=	1.7056179
	Df	=	26
	Total sample size	=	27
	Actual power	=	0.8118316

Based on this information, we should aim for a sample of N=30.

References

1. Savin, Z., Kivity, S., Yonath, H., & Yehuda, S. (2018). Smoking and the intestinal microbiome. *Archives of microbiology*, *200*(5), 677-684.
2. Redondo, N., Villavisencio, B., Díaz, L. E., Marcos, A., & Nova, E. (2017). Effect of different amounts of alcohol intake on the gut microbiota composition in healthy adults from two European countries.
3. Stadlbauer, V., Horvath, A., Komarova, I., Schmerboeck, B., Feldbacher, N., Wurm, S., ... & Stryeck, S. (2019). A single alcohol binge impacts on neutrophil function without changes in gut barrier function and gut microbiome composition in healthy volunteers. *PloS one*, *14*(2).
4. Papalini, S., Michels, F., Kohn, N., Wegman, J., van Hemert, S., Roelofs, K., ... & Aarts, E. (2019). Stress matters: randomized controlled trial on the effect of probiotics on neurocognition. *Neurobiology of stress*, *10*, 100141.
5. Allen, A. P., Hutch, W., Borre, Y. E., Kennedy, P. J., Temko, A., Boylan, G., ... & Clarke, G. (2016). Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. *Translational psychiatry*, *6*(11), e939-e939.
6. Bloemendaal, M., Szopinska-Tokov, J., Boverhof, D., Papalini, S., Michels, F., Arias-Vasquez, A., & Aarts, E. (2019). PROBIOTIC-INDUCED CHANGES IN GUT MICROBIAL COMPOSITION RELATE TO ITS BUFFERING EFFECT AGAINST THE NEGATIVE CONSEQUENCES OF STRESS ON COGNITIVE PERFORMANCE. *EUROPEAN NEUROPSYCHOPHARMACOLOGY*, *29*, S251-S252.
7. Chung, Y. C., Jin, H. M., Cui, Y., Jung, J. M., Park, J. I., Jung, E. S., ... & Chae, S. W. (2014). Fermented milk of Lactobacillus helveticus IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *Journal of functional foods*, *10*, 465-474.
8. Allen, A. P., Hutch, W., Borre, Y. E., Kennedy, P. J., Temko, A., Boylan, G., ... & Clarke, G. (2016). Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. *Translational psychiatry*, *6*(11), e939-e939.
9. Senan, S., Prajapati, J. B., Joshi, C. G., Sreeja, V., Gohel, M. K., Trivedi, S., ... & Patel, H. A. (2015). Geriatric respondents and non-respondents to probiotic intervention can be differentiated by inherent gut microbiome composition. *Frontiers in microbiology*, *6*, 25.
10. Volokh, O., Klimenko, N., Berezhnaya, Y., Tyakht, A., Nesterova, P., Popenko, A., & Alexeev, D. (2019). Human gut microbiome response induced by fermented dairy product intake in healthy volunteers. *Nutrients*, *11*(3), 547.
11. Mobini, R., Tremaroli, V., Ståhlman, M., Karlsson, F., Levin, M., Ljungberg, M., ... & Jansson, P. A. (2017). Metabolic effects of L actobacillus reuteri DSM 17938 in people with type 2 diabetes: A randomized controlled trial. *Diabetes, Obesity and Metabolism*, *19*(4), 579-589.
12. Bagga, D., Reichert, J. L., Koschutnig, K., Aigner, C. S., Holzer, P., Koskinen, K., ... & Schöpf, V. (2018). Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes*, *9*(6), 486-496.
13. Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., ... & Mayer, E. A. (2013). Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology*, *144*(7), 1394-1401.
14. Rudzki, L., Ostrowska, L., Pawlak, D., Małus, A., Pawlak, K., Waszkiewicz, N., & Szulc, A. (2019). Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: A double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology*, *100*, 213-222.
15. Ceccarelli, G., Brenchley, J. M., Cavallari, E. N., Corano Scheri, G., Fratino, M., Pinacchio, C., ... & Vullo, V. (2017). Impact of high-dose multi-strain probiotic supplementation on

neurocognitive performance and central nervous system immune activation of HIV-1 infected individuals. *Nutrients*, *9*(11), 1269.

16. Wallis, A., Ball, M., Butt, H., Lewis, D. P., McKechnie, S., Paull, P., ... & Bruck, D. (2018). Open-label pilot for treatment targeting gut dysbiosis in myalgic encephalomyelitis/chronic fatigue syndrome: neuropsychological symptoms and sex comparisons. *Journal of translational medicine*, *16*(1), 24.
17. Chung, Y. C., Jin, H. M., Cui, Y., Jung, J. M., Park, J. I., Jung, E. S., ... & Chae, S. W. (2014). Fermented milk of Lactobacillus helveticus IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *Journal of functional foods*, *10*, 465-474.
18. Hwang, Y. H., Park, S., Paik, J. W., Chae, S. W., Kim, D. H., Jeong, D. G., ... & Jung, S. J. (2019). Efficacy and safety of Lactobacillus plantarum c29-fermented soybean (dw2009) in individuals with mild cognitive impairment: a 12-week, multi-center, randomized, double-blind, placebo-controlled clinical trial. *Nutrients*, *11*(2), 305.
19. Owen, L., Reinders, M., Narramore, R., Marsh, A. R., Lui, F. G., Baron, R., ... & Corfe, B. M. (2014). A double blind, placebo controlled, randomised pilot trial examining the effects of probiotic administration on mood and cognitive function. *Proceedings of the Nutrition Society*, *73*(OCE1).
20. Kelly, J. R., Allen, A. P., Temko, A., Hutch, W., Kennedy, P. J., Farid, N., ... & Clarke, G. (2017). Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain, behavior, and immunity*, *61*, 50-59.
21. 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.
22. Chahwan, B., Kwan, S., Isik, A., van Hemert, S., Burke, C., & Roberts, L. (2019). Gut feelings: a randomised, triple-blind, placebo-controlled trial of probiotics for depressive symptoms. *Journal of affective disorders*, *253*, 317-326.
23. Rao, A. V., Bested, A. C., Beaulne, T. M., Katzman, M. A., Iorio, C., Berardi, J. M., & Logan, A. C. (2009). A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut pathogens*, *1*(1), 6.
24. Smith-Ryan, A. E., Mock, M. G., Trexler, E. T., Hirsch, K. R., & Blue, M. N. (2019). Influence of a multistrain probiotic on body composition and mood in female occupational shift workers. *Applied Physiology, Nutrition, and Metabolism*, *44*(7), 765-773.
25. Smith, A. P., Sutherland, D., & Hewlett, P. (2015). An investigation of the acute effects of oligofructose-enriched inulin on subjective wellbeing, mood and cognitive performance. *Nutrients*, *7*(11), 8887-8896.
26. Best, T., Howe, P., Bryan, J., Buckley, J., & Scholey, A. (2015). Acute effects of a dietary non-starch polysaccharide supplement on cognitive performance in healthy middle-aged adults. *Nutritional neuroscience*, *18*(2), 76-86.
27. Abildgaard, A., Elfving, B., Hokland, M., Wegener, G., & Lund, S. (2017). Probiotic treatment reduces depressive-like behaviour in rats independently of diet. *Psychoneuroendocrinology*, *79*, 40-48.
28. Wallis, A., Ball, M., Butt, H., Lewis, D. P., McKechnie, S., Paull, P., ... & Bruck, D. (2018). Open-label pilot for treatment targeting gut dysbiosis in myalgic encephalomyelitis/chronic fatigue syndrome: neuropsychological symptoms and sex comparisons. *Journal of translational medicine*, *16*(1), 24.