Integral Solutions

SCIENTIFIC REPORT

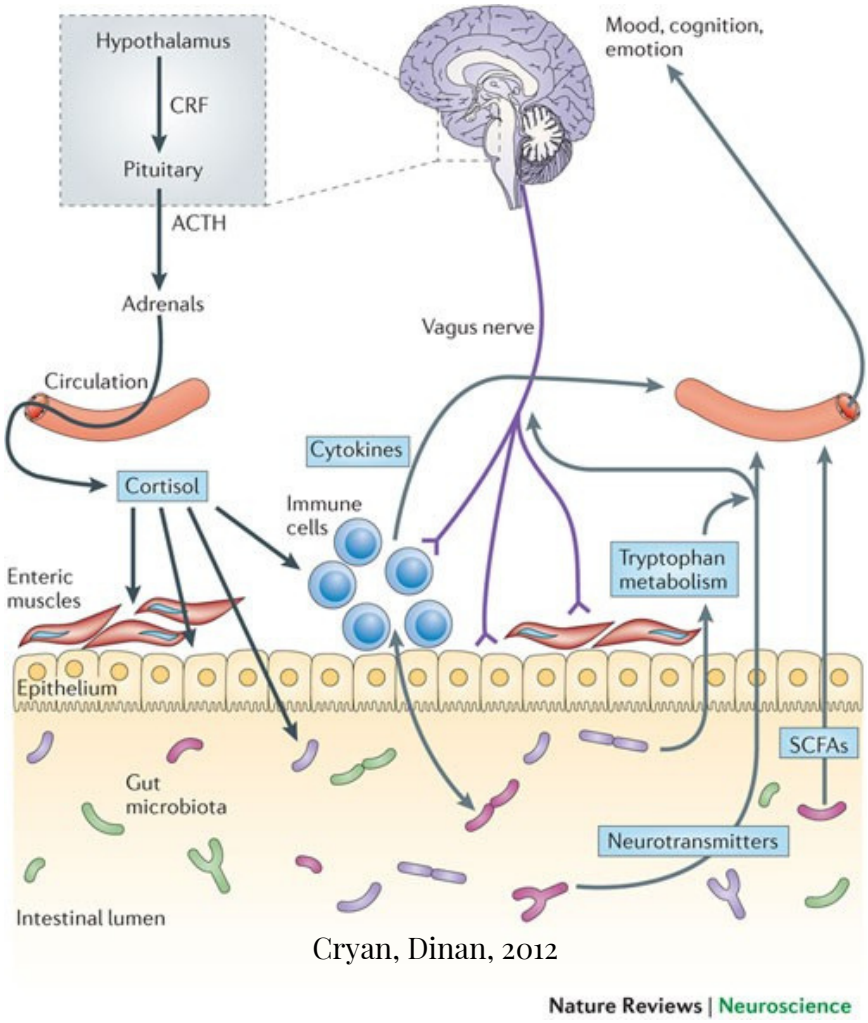
INTEGRAL BIOTICS® EASYMIND™

TABLE OF CONTENTS

GUT-BRAIN AXIS

The gut-brain axis is a bidirectional communication system that integrates neural, hormonal, immunological and metabolic signalling between the gut and the brain (Mayer, 2011). Information on neural, hormonal changes, as well as changes within the microbiota, are delivered to the brain via the vagus nerve that is a direct link between the brain and the gut (Forsythe et al., 2014).

The term "gut-brain-axis" describes an integrative physiology concept that incorporates all, including afferent and efferent neural, endocrine, nutrient, and immunological signals between CNS and the gastrointestinal system. Mechanisms by which bacteria access the brain and influence behaviour include bacterial products that gain access to the brain via the bloodstream and the area postrema, via cytokine release from mucosal immune cells, via the release of gut hormones such as serotonin from enteroendocrine cells, or via afferent neural pathways, including vagus nerve (Collins et al., 2012).



Cryan, Dinan, 2012

NERVOUS SYSTEM

Microbiome exerts its action through enteric nervous system (ENS), that governs the gastrointestinal functions and vagal afferent nerves, that convey sensory information from viscera to the CNS. The ENS is the largest and most complex division of the peripheral and autonomic nervous systems. It contains many different types of neurons comparable in number to that of the spinal cord and an array of neurotransmitters and neuromodulators similar to those found in the central nervous system (CNS) (Sasselli et al., 2012). Both the vagus nerve and modulation of systemic tryptophan levels are strongly implicated in the influence that the gut microbiota has on the brain.

Bacteria have the capacity to generate many neurotransmitters and neuromodulators. It has been determined that selective psychobiotic bacteria strains have an ability to produce serotonin, dopamine, acetylcholine or GABA (Barrett et al., 2012; Lyte, 2011; Matur, Eraslan, 2012). Psychobiotics modulate the concentrations of opioid and cannabinoid receptors in the gut epithelium (Rousseaux et al., 2007). It is conceivable that secreted neurotransmitters of microorganisms in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signalling within the enteric nervous system, or act directly on primary afferent axons (Forsythe, Kunze, 2012).

NEUROENDOCRINE SYSTEM

In the endocrinal pathway, the gut microbiome plays a major role in the development and regulation of the hypothalamus-pituitary-adrenal (HPA) axis that is critical to stress response and endocrine cells secrete neurotransmitters in response to luminal stimuli, acting as transducers for the gut-endocrine-CNS route (Rhee et al., 2009).

The HPA axis regulates cortisol secretion that can affect immune cells (including cytokine secretion) both locally in the gut and systemically. Cortisol can also alter gut permeability and barrier function, and change gut microbiota composition (Cryan, Dinan, 2012).

In addition to altering the gut microbiota composition, it is important to note that chronic stress also disrupts the intestinal barrier, making it leaky and increasing the circulating levels of immunomodulatory bacterial cell wall components such as lipopolysaccharide (Santos et al., 2001; Soderholm, Perdue, 2001). These effects can be reversed by probiotic agents (Ait-Belgnaoui et al., 2012; Zareie et al., 2006). In line with these findings, human studies show increased bacterial translocation in stress-related psychiatric disorders such as depression (Maes et al., 2012).

Approaches that have been used to elucidate the role of the gut microbiota on behaviour and cognition include the use of germ-free animals, animals with pathogenic bacterial infections, and animals exposed to probiotic agents or to antibiotics (Cryan, O'Mahony, 2011). Most of these studies highlight a role for the microbiota in modulating the stress response and in modulating stress-related behaviours that are relevant to psychiatric disorders such as anxiety and depression (Cryan, Dinan, 2012).

IMMUNE SYSTEM

The immunological pathway is an independent mechanism in the microbiome-gut-CNS signalling. Immune-to-CNS communication is mediated by systemic circulation of immune factors, which is implicated in neuropsychiatric disorders such as depression. Microbiota plays an important role in immune system regulation via anti-inflammatory mechanisms that can counteract immunemediated CNS disease symptoms (Berer, Krishnamoorthy, 2012). The gut microbiota and probiotic agents can alter the levels of circulating cytokines, and this can have a marked effect on brain function (Cryan, Dinan, 2012).

Microbiota and probiotic agents can have direct effects on the immune system (Duerkop, Vaishnava, Hooper, 2009; Forsythe, Bienenstock, 2010). Indeed, the innate and adaptive immune system collaborate to maintain homeostasis at the luminal surface of the intestinal host-microbial interface, which is crucial for maintaining health (Duerkop et al., 2009). The immune system also exerts a bidirectional communication with the CNS (Dantzer et al., 2008; Sternberg, 2006) making it a prime target for transducing the effects of bacteria on the CNS. In addition, indirect effects of the gut microbiota and probiotics on the innate immune system can result in alterations in the circulating levels of pro-inflammatory and anti-inflammatory cytokines that directly affect brain function (Cryan, Dinan, 2012).

METABOLIC PATHWAY

Gut bacteria modulate various host metabolic reactions, resulting in the production of metabolites such as secondary bile acids, choline and short-chain fatty acids that are essential for host health (Nicholson et al., 2012). Indeed, complex carbohydrates such as dietary fibre can be digested and subsequently fermented in the colon by gut microorganisms into short-chain fatty acids such as n-butyrate, acetate and propionate, which are known to have neuroactive properties and can modulate brain and behaviour (Gundersen, Blendy, 2009; MacFabe et al., 2011; Thomas et al., 2012).

HOW DOES EASYMIND™ WORK?

It has been demonstrated that probiotics have broader therapeutic applications than previously considered.

Microbiota-derived products are components responsible for the microbiota-gut-CNS signalling. Some bacteria strains can secrete different neurotransmitters needed for regulation of many psychological processes. It has been demonstrated that bacteria contained in Integral Biotics EasyMind™ have an ability to produce: GABA, dopamine or acetylcholine, which can help patients suffering from psychiatric illness, such as depression or anxiety.

GABA (γ-Aminobutyric acid) is the main inhibitory neurotransmitter in the CNS, and plays a central role in regulating many physiological and psychological processes. Dysfunction of the central gamma-aminobutyric system has long been associated with anxiety disorders (Lydiard, 2003; Möhler, 2012; Nemeroff, 2003; Nutt, Malizia, 2001). GABA, as a part of both anxiety and depression pathogenesis, is responsible for many symptoms of these disorders (Kalueff, Nutt, 2007). Activation of the GABA-ergic system has both anxiolytic and antidepressant effects in animals and humans. In contrast, decreased GABAergic activity consistently correlates with anxiety and depression. Together, this clearly demonstrates the key role that GABA plays in both psychopathologies and indicates that products affecting GABA-A receptors may be useful in the treatment of both anxiety and depression. (Kalueff, Nutt, 2007).

Dopamine is a complex neurotransmitter that can create a variety of health problems if levels are either too high or too low (Worley, 2017) Some of the vital functions that dopamine plays a role in include movement, memory, pleasure, attention, mood, sleep, behaviour, and cognition, as well as regulation of prolactin levels (Calabresi et al., 2007).

Abnormally low levels of this neurotransmitter are associated with attention deficits, disorganised thinking, poor concentration, depression. According to this, it has been observed that lower levels of dopamine are associated with symptoms of attention deficit hyperactivity disorder (ADHD) (Wu et al., 2012).

Emerging data have also linked dopamine system dysfunction to the pathophysiology of depression (Chaudhury et al., 2013). Serotonin has traditionally been the transmitter linked with depression, based on pharmacological studies of antidepressant drugs that target the serotonin system or depletion of serotonin in the CNS. However, many of the symptoms seen in depression- such as anhedonia and lack of motivation- have been more consistently associated with dysfunctions in the dopamine system (Eschel et al., 2016; Grace, 2016; Pandit et al., 2016). It has been reported that dopamine depletion would be the inducer of anxiety-like behaviours as well (DeGroot et al., 2020).

Acetylcholine is a neurotransmitter found in the central and peripheral nervous systems. Acetylcholine has numerous functions in the body. It can be found in all motor neurons, where it stimulates muscles to contract (Sam, Bordoni, 2021). From the movements of the stomach to the blink of an eye, all of the body's movements involve the activity of this significant neurotransmitter.

It is also found in many brain neurons and plays a critical role in mental processes, such as memory, learning and cognition (Picciotto et al., 2012). Acetylcholine also acts at various sites within the CNS, where it can function as a neurotransmitter and as a neuromodulator. It plays a role in motivation, arousal, attention, learning, and memory, and is also involved in promoting REM sleep (Picciotto et al., 2012).

Moreover, gut permeability has been directly and indirectly associated with the role of microbiome in CNS disorders. Humoral and cellular immune reaction to microbiota in the circulation, persistent low-grade inflammation and neuropsychiatric comorbidity with IBD may hint the breach of mucosal epithelial barrier (Maes et al., 2012; Severance et al., 2013).

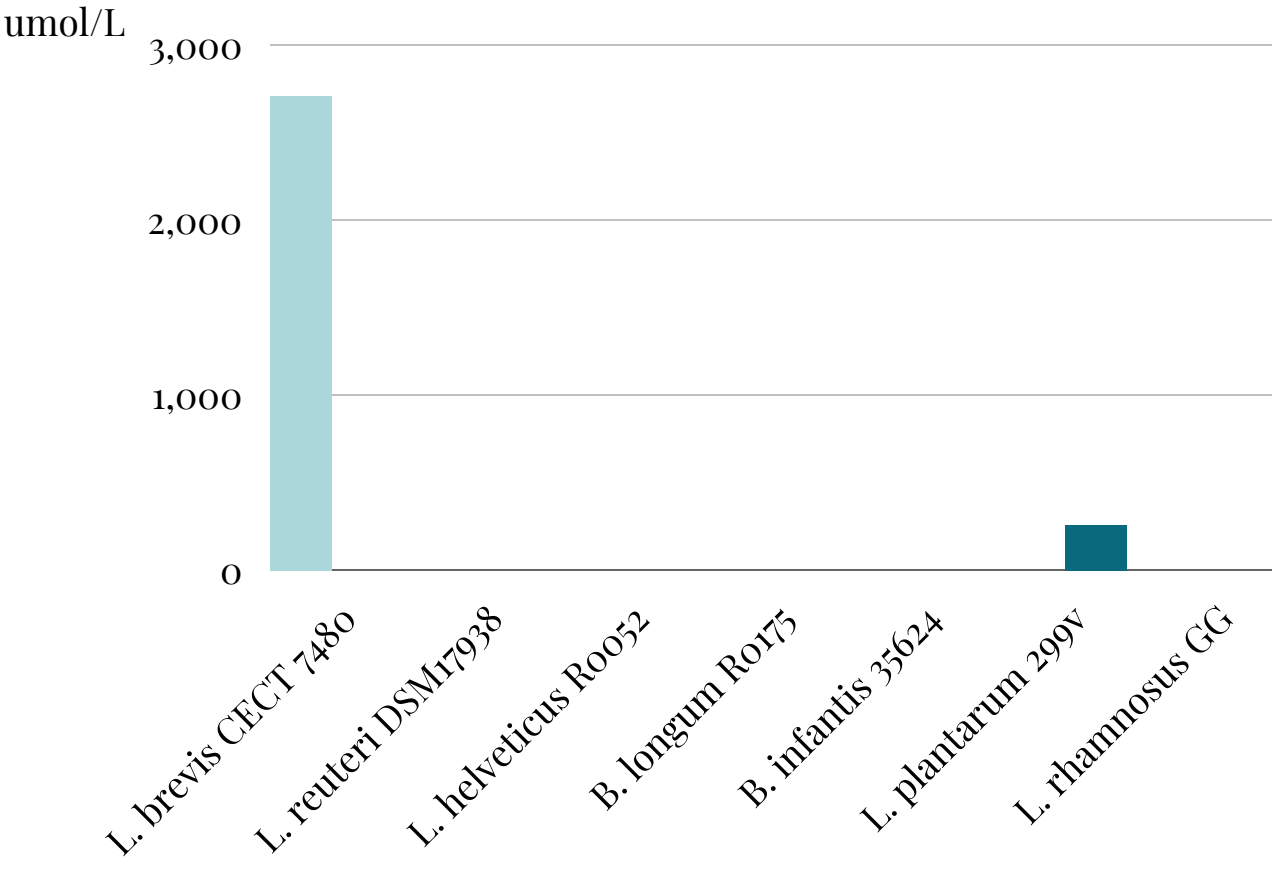
Microbiota and their ligand as polyphosphate granules maintain the cell-cell junctions critical to barrier integrity and decrease CNS pathogenesis (Segawa et al., 2011).

Our partners have carried out a series of analyses among their large culture collection, in order to specifically select the most efficient bacterial strains in the production of the neurotransmitters GABA, dopamine and acetylcholine. Two *Lactobacillus* species were chosen:

- L*actobacillus brevis* CECT 7480: Found to produce the highest levels of GABA and dopamine among several well-known psychobiotics which were used as controls (*L. rhamnosus* GG, *B. longum/infantis* 35624, *L. reuteri* DSM17938, *L. helveticus* R0052 and *B. longum* R0175).

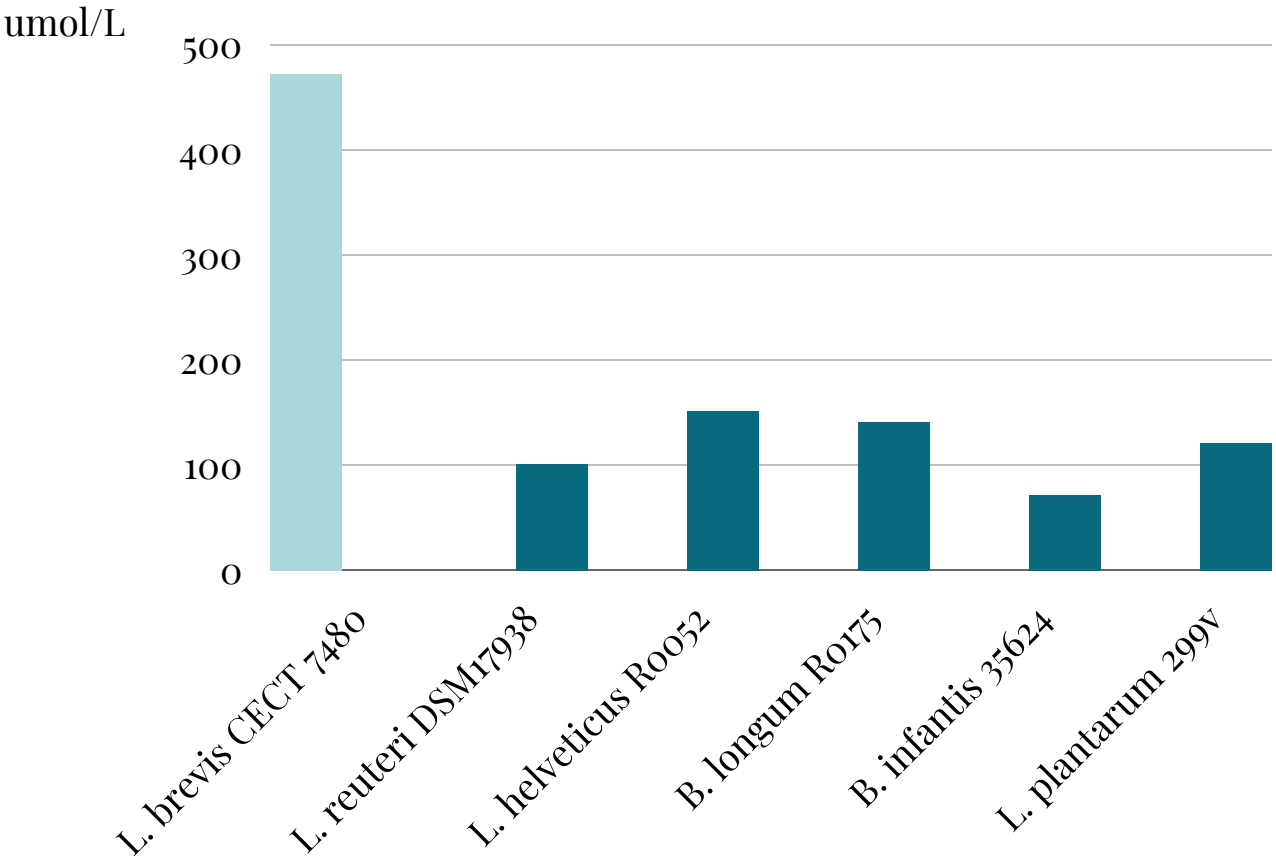
L. helveticus R0052 and *B. longum* R0175 are well known strains that are contained in Probio'Stick by Lallemand Health Solutions- the most studied psychobiotic worldwide.

GABA PRODUCTION BY EASYMIND™



GABA constitutes a major inhibitory neurotransmitter in the sympathetic nervous system and has antidepressant, antihypertensive, and anti-diabetic effects in humans (Wu et al., 2018). Oral intake of GABA is associated with reduced stress and better ability to focus on priority tasks (Mazzoli, Pessione, 2016), and the abundance of GABA-producing bacteria in the human gut is inversely correlated to brain signatures associated with depression (Strandwitz et al., 2016). Therefore, GABA has been classified as a bioactive component in foods and pharmaceuticals.

DOPAMINE PRODUCTION BY EASYMIND™

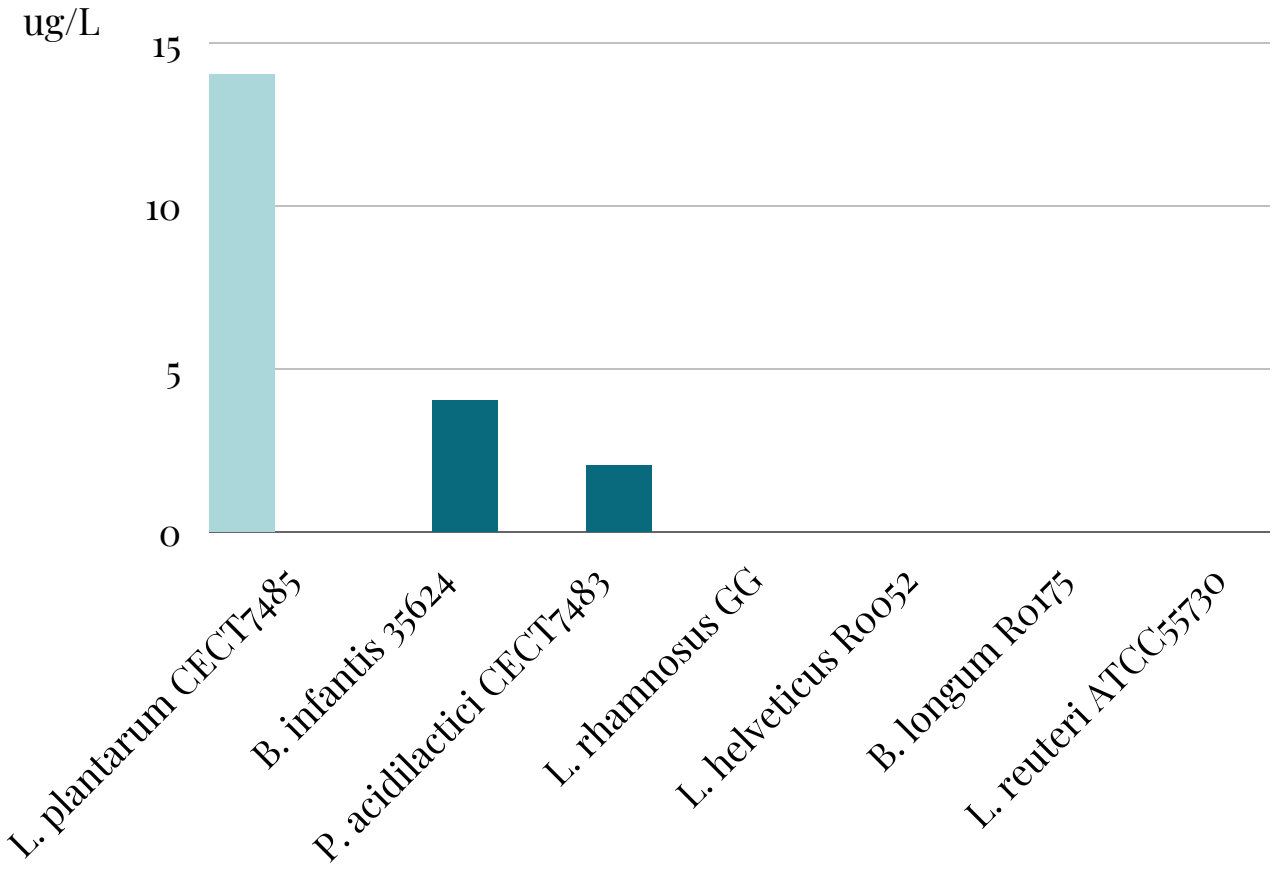


The mitigation of low-grade inflammation is one of the mechanisms for psychobiotic effects that is typically observed as reductions in circulating pro-inflammatory cytokines concentrations (Sarkar et al., 2016). Proinflammatory cytokines are also capable of increasing the permeability of the blood–brain barrier, permitting access to potential pathogenic entities.

Cytokines alter concentrations of several neurotransmitters that regulate communication in the brain, including serotonin, dopamine, and glutamate (Sarkar et al., 2016). Considering this fact, we also choose this strain for its high production of dopamine, a neurotransmitter that regulates many processes, including reward, motivation, and addiction. CNS disorders, such as depression, anxiety, schizophrenia, bipolar disorder, and Parkinson's disease, have been linked to dopamine dysregulation (Berk et al., 2007, Weintraub et al., 2005).

- *Lactobacillus plantarum* CECT 7485: this strain has been selected to produce high levels of acetylcholine. In addition to its well-known effect on muscle contractility (via muscarinic receptors), acetylcholine is a chief anti-inflammatory neurotransmitter in the gut mucosa via cholinergic anti-inflammatory pathway, an efferent vagus nerve-based mechanism (Rosas-Ballina, Tracey, 2009).

ACETYLCHOLINE PRODUCTION BY EASYMIND™



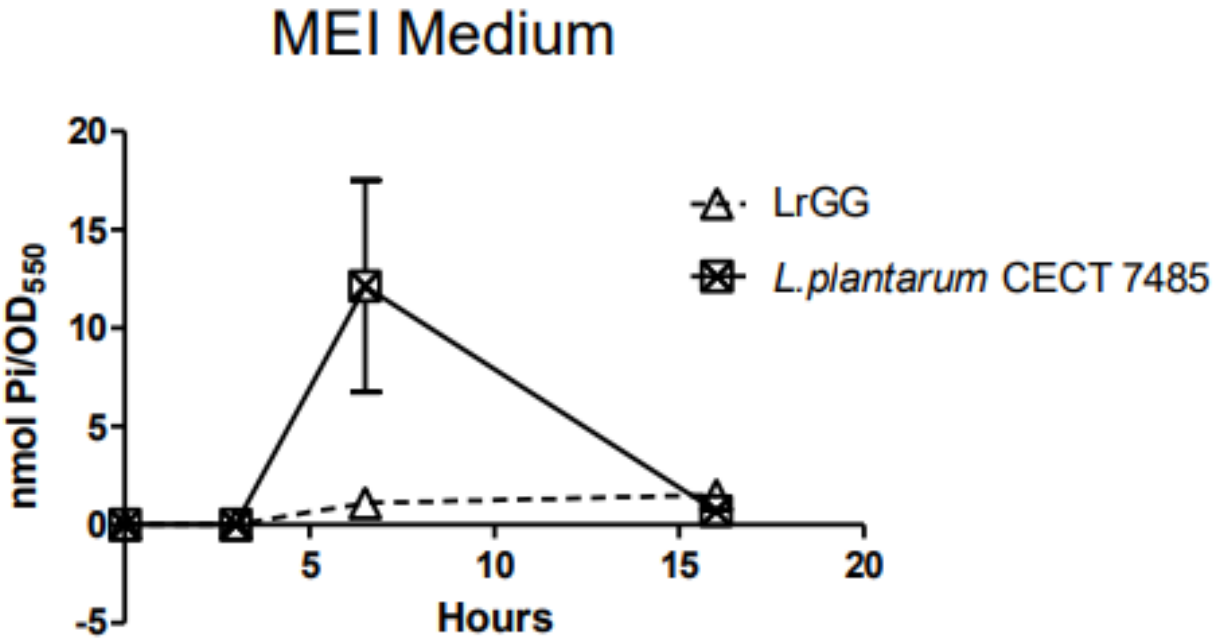
In the cholinergic anti-inflammatory pathway, the efferent arm of the inflammatory reflex, is composed of the vagus nerve, the neurotransmitter acetylcholine and the α7 subunit of the nicotinic acetylcholine receptor. Acetylcholine attenuates the production of TNF, IL-1β, IL6 and IL-18 by human macrophages at the post-transcriptional stage, indicating an inhibitory effect of acetylcholine on pro-inflammatory cytokine production (Borovikova et al., 2000).

Stimulating the vagus nerve exerts anti-inflammatory effects via acetylocholine that is currently used as a therapy on refractory depression, pain and epilepsy (Sarkar et al., 2016). Therefore, probiotics that have a high production of this neurotransmitter, are desirable by exerting an anti-inflammatory function through vagal nerve modulation, complementing the concomitant effect of antidepressant and anxiolytic drugs (Sarkar et al., 2016). Moreover, neuroinflammation is increasingly linked to adverse memory, especially in Alzheimer's disease and disruption of cholinergic circuitry is likely to be at least partly responsible for the cognitive impairments seen in neurodegenerative disorder (Maurer, Williams, 2017). It have been showed that cholinergic degeneration and deficit of acetylcholine is life-threatening in creation of the symptoms of AD (Nimgampalle, Kuna, 2017; Terry, Buccafusco, 2003).

Moreover, L. plantarum CECT 7485 also contains poly-phosphate granules. Inorganic polyphosphates (poly-P) consist of linear chains of hundreds of phosphate molecules linked by phosphoanhydride bonds. Their presence in lactic acid bacteria is a strain-specific trait with high variability between different strains, and is thought to help some strains cope with oxidative and osmotic stress (Alcantara et al., 2014). It has been showed that the release of soluble poly-P granules into the medium enhance the barrier function and reduce inflammatory reactions in the gut epithelium in vitro and in vivo (Kashima et al., 2015; Segawa et al., 2011). Detailed studies showed the effect to be dependent on integrin β1- mediated capture of soluble poly-P in the cell membrane, followed by caveolin-1-mediated endocytosis.

This results in MAPK p38-mediated cytoprotective effects in the epithelial cells, such as induction of heat shock protein 27 (Tanaka et al., 2015).

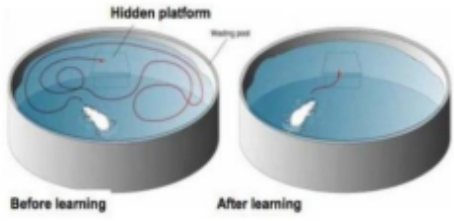
The Lactobacillus plantarum CECT 7485 strain presents a capacity of the production of polyphosphates 10 times greater than other Lactobacillus strains like Lactobacillus rhamnosus GG, at the same point of growth. This property a desirable feature in a probiotic for preventing and alleviating intestinal inflammation and improves the intestinal barrier function.

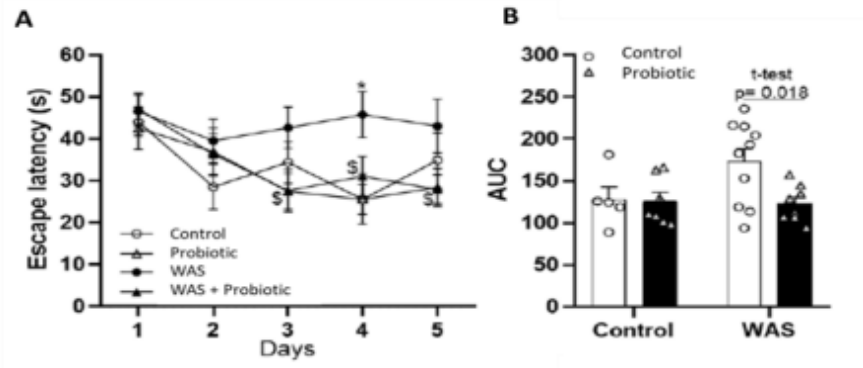


PRE- AND CLINICAL STUDIES ON EASYMIND™

PRE-CLINICAL TRIAL IN ANIMAL MODELS

AIM	To demonstrate that EasyMind™ psychobiotic formula enhances cognitive performance under stressful situations.
DESIGN	Experimental mouse model of induced cognitive deficit by "water avoidance stress" test (WAS).
RESULTS	Probiotic formula was able to significantly revert the cognitive dysfunction from day 3 onwards. Therefore, probiotic improved cognitive function under stress/anxiety situation.

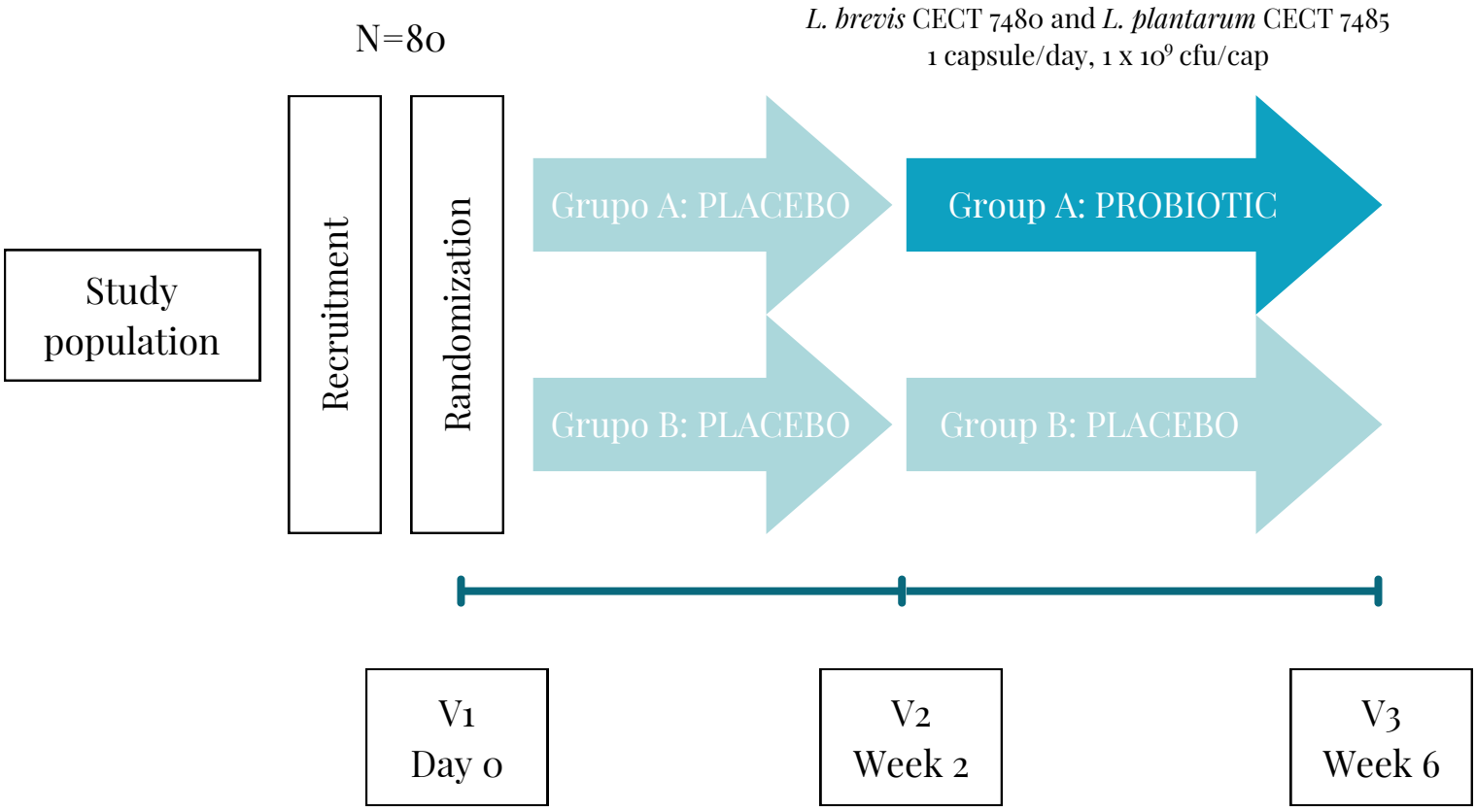




[$: P<0.05 compared to control]

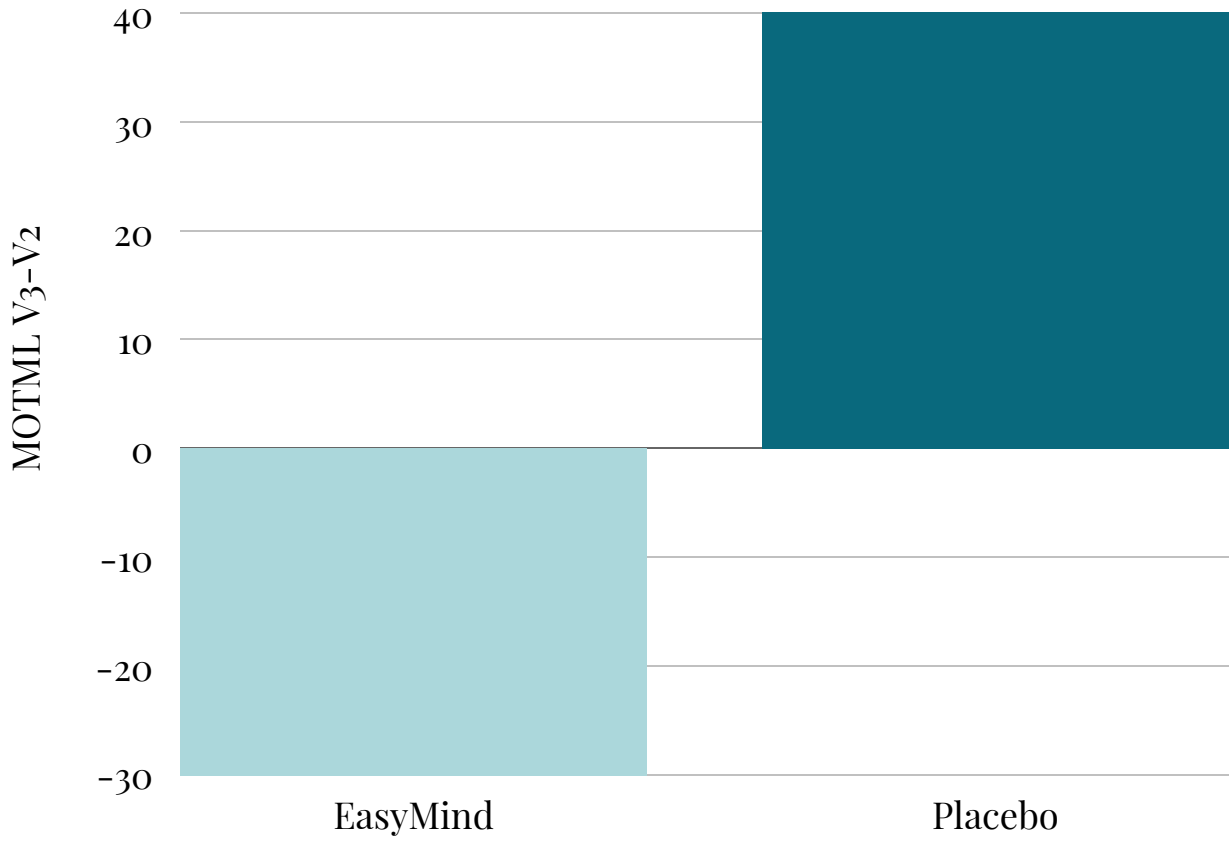
CLINICAL TRIAL ON COGNITION, STRESS & ANXIETY

AIM	To demonstrate that EasyMind™ psychobiotic formula enhances concentration, learning and memorization.
DESIGN	Randomized, placebo-controlled, double-blind study.
POPULATION	80 adults divided into 2 groups: probiotic group (n=40) and placebo group (n=40).
TREATMENT	Probiotic/placebo daily administration during 4 weeks.
PRIMARY ENDPOINT	Cognitive function evaluated through CANTAB (Cambridge Neuropsychological Test Automated Battery), performed after a Trier Social Stress Test (TSST)
SECONDARY ENDPOINT	a) Stress: Perceived stress scale (PSS), visual analog scale (VAS) and cortisol, b) Depression, anxiety and stress scales (DASS-21), Profile of moods states (POMS), Positive and negative scale (PANAS), c) Quality of life (SF12) and sleep quality. Anthropometric and socio-demographic data and medical history collected



RESULTS	80 participants finalized the follow-up. Demographic variables at baseline did not differ between probiotic and placebo groups. Stress scores evaluated by PSS were medium to low and there were no differences between groups. During TSST protocol, cortisol levels increased in both groups, but no significant differences were observed between them. After TSST, MOTML values were significantly lower in the probiotic than in placebo group (-31.72 vs 37.84 ; p=0.0137). Increase in cortisol concentrations during TSST were positively correlated with MOTML in the control group (r=0.45, p=0.001), but not in the probiotic group (r=0.113, p=0.47).

MOTML SCORES IN PROBIOTIC AND PLACEBO GROUP



CONCLUSIONS	The results from this study suggest that probiotic formulation in a healthy population subjected to a stressful situation may have positive effects in psychomotor function and attention. The improvement in psychomotor speed and accuracy could be considered as a cognitive protector.

CLINICAL TRIAL IN INFANTS WITH ADHD AND ASD

AIM	To investigate the epidemiology of autism spectrum disorders (ASD), social (pragmatic) and attention deficit hyperactivity disorder (ADHD) and its relation to nutritional and environmental factors in a school population in Spain (4-14 years old).
DESIGN	Randomized, placebo-controlled, double-blind study.
POPULATION	40 children diagnosed with ASD (n=20 placebo and n=20 active) and 40 children diagnosed with ADHD (n=20 placebo and n=20 active). Diagnosed according to DSM-V criteria bystandardized interviews with parents and children.
ASSESSMENT	Anthropometric and nutritional assessment. Socio-demographic data and medical history collected.
TREATMENT	Probiotic/placebo daily administration during 3 months.
PRIMARY ENDPOINTS	Changes in the severity of symptoms.
STATUS OF THE STUDY	Patient follow-up finalized. Now creating study database to perform statistical analysis.

SUMMARY

- Intestinal dysbiosis is associated with CNS disorders such depression or anxiety.
- GABA dysfunction is associated with anxiety and depression. Low levels of dopamine are associated with symptoms of ADHD and neurodegenerative disorders.
- EasyMind's™ unique strain L. brevis CECT 7480 has been specifically selected for its ability to produce the neurotransmitters GABA and dopamine.
- EasyMind's™ unique strain L. plantarum CECT 7485 has been specifically selected for its ability to produce acetylcholine and poly-phosphate granules to enhance the barrier function and reduce inflammation in the gut epithelium.
- Psychobiotics such as EasyMind™ are live microorganisms that may produce a health benefit in psychiatric patients.

REFERENCES

1. Alcántara, C., Blasco, A., Zúñiga, M., & Monedero, V. (2014). Accumulation of polyphosphate in Lactobacillus spp. and its involvement in stress resistance. Applied and environmental microbiology, 80(5), 1650–1659. https://doi.org/10.1128/AEM.03997-13
2. Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., ... & Theodorou, V. (2012). Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology, 37(11), 1885-1895.
3. Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., & Stanton, C. (2012). γ-Aminobutyric acid production by culturable bacteria from the human intestine. Journal of applied microbiology, 113(2), 411-417.
4. Berer, K., & Krishnamoorthy, G. (2012). Commensal gut flora and brain autoimmunity: a love or hate affair?. Acta neuropathologica, 123(5), 639-651.
5. Berk, M., Dodd, S., Kauer-Sant'anna, M., Malhi, G. S., Bourin, M., Kapczinski, F., & Norman, T. (2007). Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. Acta Psychiatrica Scandinavica, 116, 41-49.
6. Berry, A. S., White, R. L., Furman, D. J., Naskolnakorn, J. R., Shah, V. D., D'Esposito, M., & Jagust, W. J. (2019). Dopaminergic mechanisms underlying normal variation in trait anxiety. Journal of Neuroscience, 39(14), 2735-2744.
7. Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., ... & Tracey, K. J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature, 405(6785), 458-462.
8. Calabresi, P., Picconi, B., Tozzi, A., & Di Filippo, M. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends in neurosciences, 30(5), 211–219. https://doi.org/10.1016/j.tins.2007.03.001
9. Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., ... & Han, M. H. (2013). Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature, 493(7433), 532-536.
10. Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. Nature Reviews Microbiology, 10(11), 735-742.
11. Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nature Reviews Neuroscience, 13(10), 701–712. doi:10.1038/nrn3346
12. Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. Neurogastroenterology & Motility, 23(3), 187-192.
13. Dantzer, R., O'connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. Nature reviews neuroscience, 9(1), 46-56.
14. DeGroot, S. R., Zhao-Shea, R., Chung, L., Klenowski, P. M., Sun, F., Molas, S., ... Tapper, A. R. (2020). Midbrain Dopamine Controls Anxiety-like Behavior by Engaging Unique Interpeduncular Nucleus Microcircuitry. Biological Psychiatry, 88(11), 855–866. doi:10.1016/j.biopsych.2020.06.01
15. Duerkop, B. A., Vaishnava, S. & Hooper, L. V. (2009). Immune responses to the microbiota at the intestinal mucosal surface. Immunity 31, 368–376.
6. Eshel, N., Tian, J., Bukwich, M., & Uchida, N. (2016). Dopamine neurons share common response function for reward prediction error. Nature neuroscience, 19(3), 479–486. https://doi.org/10.1038/nn.4239

17. Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. Immunological investigations, 39(4-5), 429-448.
18. Forsythe, P., & Kunze, W. A. (2013). Voices from within: gut microbes and the CNS. Cellular and molecular life sciences, 70(1), 55-69.
19. Grace, A. A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. Nature Reviews Neuroscience, 17(8), 524–532. doi:10.1038/nrn.2016.57
20. Gundersen, B. B. & Blendy, J. A. (2009). Effects of the histone deacetylase inhibitor sodium butyrate in models of depression and anxiety. Neuropharmacology 57, 67–74.
21. Kalueff, A. V., & Nutt, D. J. (2007). Role of GABA in anxiety and depression. Depression and anxiety, 24(7), 495-517.
22. Kashima, S., Fujiya, M., Konishi, H., Ueno, N., Inaba, Y., Moriichi, K., ... & Kohgo, Y. (2015). Polyphosphate, an active molecule derived from probiotic Lactobacillus brevis, improves the fibrosis in murine colitis. Translational Research, 166(2), 163-175.
23. Lydiard, R. B. (2003). The role of GABA in anxiety disorders. Journal of Clinical Psychiatry, 64, 21-27.
24. Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. Bioessays 33, 574–581.
25. MacFabe, D. F., Cain, N. E., Boon, F., Ossenkopp, K. P., & Cain, D. P. (2011). Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder. Behavioural brain research, 217(1), 47-54.
26. Maes, M., Twisk, F. N., Kubera, M., Ringel, K., Leunis, J. C., & Geffard, M. (2012). Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. Journal of affective disorders, 136(3), 909-917.
27. Matur, E., & Eraslan, E. (2012). The impact of probiotics on the gastrointestinal physiology. New advances in the basic and clinical gastroenterology, 1, 51-74.
28. Maurer, S. V., & Williams, C. L. (2017). The cholinergic system modulates memory and hippocampal plasticity via its interactions with non-neuronal cells. Frontiers in immunology, 8, 1489.
29. Mayer, E. A. (2011). Gut feelings: the emerging biology of gut–brain communication. Nature Reviews Neuroscience, 12(8), 453-466.
30. Mazzoli, R., & Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. Frontiers in microbiology, 7, 1934.
31. Möhler, H. (2012). The GABA system in anxiety and depression and its therapeutic potential. Neuropharmacology, 62(1), 42-53.
32. Nemeroff C. B. (2003). The role of GABA in the pathophysiology and treatment of anxiety disorders. Psychopharmacology bulletin, 37(4), 133–146.
33. Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012). Host-gut microbiota metabolic interactions. Science, 336(6086), 1262-1267.
34. Nimgampalle, M., & Kuna, Y. (2017). Anti-Alzheimer properties of probiotic, Lactobacillus plantarum MTCC 1325 in Alzheimer's disease induced albino rats. Journal of clinical and diagnostic research: JCDR, 11(8), KC01.
35. Nutt, D. J., & Malizia, A. L. (2001). New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. The British journal of psychiatry: the journal of mental science, 179, 390–396. https://doi.org/10.1192/bjp.179.5.390.

36. Pandit, R., Omrani, A., Luijendijk, M., de Vrind, V. A., Van Rozen, A. J., Ophuis, R. J., ... & Adan, R. A. (2016). Melanocortin 3 receptor signaling in midbrain dopamine neurons increases the motivation for food reward. Neuropsychopharmacology, 41(9), 2241-2251.
37. Picciotto, M. R., Higley, M. J., & Mineur, Y. S. (2012). Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron, 76(1), 116-129.
38. Rhee, S. H., Pothoulakis, C., & Mayer, E. A. (2009). Principles and clinical implications of the brain–gut–enteric microbiota axis. Nature reviews Gastroenterology & hepatology, 6(5), 306-314.
39. Rosas-Ballina, M., & Tracey, K. J. (2009). Cholinergic control of inflammation. Journal of internal medicine, 265(6), 663-679.
40. Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., ... & Desreumaux, P. (2007). Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. Nature medicine, 13(1), 35-37.
41. Sam, C., & Bordoni, B. (2022). Physiology, Acetylcholine. In StatPearls [Internet]. StatPearls Publishing.
42. Santos, J., Yang, P. C., Soderholm, J. D., Benjamin, M. & Perdue, M. H. (2001). Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. Gut 48, 630–636.
43. Sarkar, A., Lehto, S. M., Harty, S., Dinan, T. G., Cryan, J. F., & Burnet, P. W. (2016). Psychobiotics and the manipulation of bacteria–gut–brain signals. Trends in neurosciences, 39(11), 763-781.
44. Segawa, S., Fujiya, M., Konishi, H., Ueno, N., Kobayashi, N., Shigyo, T., & Kohgo, Y. (2011). Probiotic-derived polyphosphate enhances the epithelial barrier function and maintains intestinal homeostasis through integrin–p38 MAPK pathway. PloS one, 6(8), e23278.
45. Severance, E. G., Gressitt, K. L., Stallings, C. R., Origoni, A. E., Khushalani, S., Leweke, F. M., ... & Yolken, R. H. (2013). Discordant patterns of bacterial translocation markers and implications for innate immune imbalances in schizophrenia. Schizophrenia research, 148(1-3), 130-137.
46. Soderholm, J. D., & Perdue, M. H. (2001). II. Stress and intestinal barrier function. American Journal of Physiology-Gastrointestinal and Liver Physiology, 280(1), G7-G13.
47. Sternberg, E. M. (2006). Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nature Reviews Immunology, 6(4), 318-328.
48. Strandwitz, P., Kim, K. H., Terekhova, D., Liu, J. K., Sharma, A., Levering, J., ... & Lewis, K. (2019). GABA-modulating bacteria of the human gut microbiota. Nature microbiology, 4(3), 396-403.
49. Tanaka, K., Fujiya, M., Konishi, H., Ueno, N., Kashima, S., Sasajima, J., ... & Kohgo, Y. (2015). Probiotic-derived polyphosphate improves the intestinal barrier function through the caveolin-dependent endocytic pathway. Biochemical and biophysical research communications, 467(3), 541-548.
50. Terry, A. V., & Buccafusco, J. J. (2003). The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug development. Journal of Pharmacology and Experimental Therapeutics, 306(3), 821-827.
51. Thomas, R. H., Meeking, M. M., Mepham, J. R., Tichenoff, L., Possmayer, F., Liu, S., & MacFabe, D. F. (2012). The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders. Journal of neuroinflammation, 9(1), 1-18.

52. Weintraub, D., Newberg, A. B., Cary, M. S., Siderowf, A. D., Moberg, P. J., Kleiner-Fisman, G., ... & Katz, I. R. (2005). Striatal dopamine transporter imaging correlates with anxiety and depression symptoms in Parkinson's disease. Journal of Nuclear Medicine, 46(2), 227-232.
53. Worley, J. (2017). The Role of Pleasure Neurobiology and Dopamine in Mental Health Disorders. Journal of Psychosocial Nursing and Mental Health Services, 55(9), 17–21. doi:10.3928/02793695-20170818-09
54. Wu, C. H., Hsueh, Y. H., Kuo, J. M., & Liu, S. J. (2018). Characterization of a potential probiotic Lactobacillus brevis RK03 and efficient production of γ-aminobutyric acid in batch fermentation. International journal of molecular sciences, 19(1), 143.
55. Wu, J., Xiao, H., Sun, H., Zou, L., & Zhu, L. Q. (2012). Role of dopamine receptors in ADHD: a systematic meta-analysis. Molecular neurobiology, 45(3), 605-620.
56. Zareie, M., Johnson-Henry, K., Jury, J., Yang, P. C., Ngan, B. Y., McKay, D. M., ... & Sherman, P. M. (2006). Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. Gut, 55(11), 1553-1560.