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TAC1 Gene Therapy in the Gut to Reduce Long-Term Memory Loss in Tg4-42 Alzheimer Diseased Mice: A Research Proposal

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Abstract
Alzheimer's disease (AD) is a neurodegenerative disorder that mainly affects a large percentage of the older adult population. AD can cause many problems, most notably long-term memory (LTM) loss. Many studies have observed a decreased level of substance P (SP) in the hippocampus of individuals with AD. In this paper, we propose a novel strategy to limit AD-related decline of LTM using probiotics transformed with SP. These transformed bacteria will contain varying concentrations of SP and will be injected into three different segments of the proximal colon in AD mouse models. LTM will be measured through Morris Water Maze (MWM) and Barnes Maze (BM) tests over three months to examine improvements in spatial memory. It is anticipated that this experiment will demonstrate that increased concentrations of SP in the proximal colon will result in the greatest reduction in LTM loss in AD individuals. This experiment will establish a new therapeutic option for AD individuals to slow the progression of LTM loss.

Keywords: Alzheimer's disease; hippocampus; substance P; Morris water maze test; *Lactobacillus rhamnosus GG*; long-term memory; Barnes maze test

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder occurring primarily in the elderly population and results in a decline in cognitive function over time [1]. The pathophysiology of this complex disease is still poorly understood; however, beta-amyloid and tau proteins are likely responsible for AD [2]. The beta-amyloid protein accumulates when its' precursor is cleaved incorrectly. This accumulation leads to fibrillary amyloids leading to neuronal damage [3]. Tau proteins are hyperphosphorylated resulting in a disruption of their function and accumulation into filaments resulting in neuronal toxicity [4]. These proteins coupled with other factors contribute to AD progression.

AD is a neurodegenerative is a neurodegenerative disorder that mainly affects a large percentage of the older adult population. AD can cause many problems, most notably long-term memory (LTM) loss. Many studies have observed a decreased level of substance P (SP) in the hippocampus of individuals with AD. In this paper, we propose a novel strategy to limit AD-related decline of LTM using probiotics transformed with SP. These transformed bacteria will contain varying concentrations of SP and will be injected into three different segments of the proximal colon in AD mouse models. LTM will be measured through Morris Water Maze (MWM) and Barnes Maze (BM) tests over three months to examine improvements in spatial memory. It is anticipated that this experiment will demonstrate that increased concentrations of SP in the proximal colon will result in the greatest reduction in LTM loss in AD individuals. This experiment will establish a new therapeutic option for AD individuals to slow the progression of LTM loss.The hippocampus is a brain region that is responsible for memory and learning [5]. The hippocampus is stimulated by SP which is an eleven amino acid long neuropeptide produced by the TAC1 gene [6]. Decreased SP levels in the hippocampus [7] have been implicated in many neurodegenerative disorders including AD [8]. SP can prevent harmful amyloid-β fragment overexpression and caspase-3-induced PARP-1 from inducing cell death, amongst many other crucial functions [9]. Furthermore, SP has been shown to induce long-term potentiation (LTP) in the hippocampi of mice [10]. Therefore, improving LTP could be used to improve LTM [11]. Given that decreased levels of SP are observed in AD individuals combined with SP's beneficial functions, this neuropeptide is an important molecule to analyze and potentially utilize as a therapeutic agent.

Current literature is sparse on what effects introducing SP into an AD model will have. We do, however, note one study that has analyzed this topic [8]. Mice with

hippocampal damage were given SP injections daily for one week. To mimic AD, the mice received an amyloid beta peptide injection in the hippocampus. Memory was then tested using the MWM. They found no correlation between escape latency in the mice receiving the SP injection versus the control group. We noted several issues with this paper. Firstly, the SP injections were transient and thus unable to produce a stable effect. Secondly, SP injections were used to determine if short-term memory was improved, but whether it also improves LTM is unclear. Finally, the mouse model utilized was given transient injections of purified amyloid proteins. This is less reflective of an AD etiology as amyloid protein concentrations are thought to slowly accumulate as the disease progresses [12] as opposed to exhibiting sudden and large accumulations mimicked by this study.

Another important consideration for an AD therapeutic target is the gut-brain axis (GBA). The GBA refers to the ongoing communication between the enteric and central nervous system [13]. The GBA is therefore important because microbial communities within the gut can influence memory [13] through the GBA. In 2016 Dr. Gradinaru's lab showed that a dysbiosis gut microbiome may contribute to the progression of Parkinson's disease [14]. This correlation between the gut microbiome and disease progression means a probiotic can be developed to reduce and/or improve the effect the microbiome has on disease outcome. To avoid complications from administering foreign microbes in the gut, native transformed gut microbes should be used to deliver therapeutic treatments. Studies have shown that common mucosa-associated bacteria such as *Lactobacillus* can be found in the proximal gut (i.e., cecum, ascending colon, and transverse colon) [15, 16]. *Lactobacilli* can adhere tightly to the epithelium of the gastrointestinal tract [17] which will allow for them to remain in the host longer. Furthermore, they are safe for human consumption and are regarded as having many beneficial properties [18]. Therefore, utilizing this microbe for the purposes of this experiment would be ideal as they will remain in the digestive tract and are safe. If this microbe were to be given TAC1 gene(s) and inoculated in the appropriate areas of the GI tract of the AD mouse model, then LTM impairment could be analyzed. While this study will ultimately require clinical trials in the future, the use of AD mouse models initially would be more ideal than human subjects since increased SP levels have been found in those with irritable bowl syndrome [19]. Thus, until a therapeutic dose is determined, exposing individuals to elevated concentrations of SP may be harmful. Furthermore, mouse models can be euthanized following each experiment allowing for examination of exact mechanisms. Since there is no current literature probing this experimental design this justifies further investigation.

Currently, there exists many AD mouse models such as the Tg2576 and 5xFAD [20, 21]. One model is the Tg4-42 AD mouse which expresses Aβ4-42 peptides (that are abundant in AD individuals) which can aggregate and become toxic to the hippocampus [22, 23]. This hippocampal neuronal degradation results in memory loss [24] hence, this model is both reflective of AD pathophysiology and demonstrates symptoms that are relevant to the therapeutic target. LTM impairment is difficult to study in mice; however, the MWM and the BM [25, 26] can asses LTM loss. These tests complement each other and reduce confounding variables as they encourage memory in mice using different stressors [27, 28] thus, increasing the likelihood that improved memory is the result of the treatment and not the stressor. This paper will propose a research protocol on how such a study could be conducted. We hypothesize that if we give Tg4-42 mice increased concentrations of SP stimulation in the proximal colon, we will reduce the progression of LTM decline.

Methods

Three plasmids will be made to contain an ampicillin gene and either one/two/ or three TAC1 genes. The plasmid will also contain a 6X histidine (HIS) tag and a strong constitutive promoter such as the rice actin 1 gene and could be designed using the 'Benchling' bioinformatics software. Three separate stock solutions of LGG cells will be transformed with our plasmids via electroporation and then will be serially diluted into MRS broth and plated on MRS agar until a single colony can be isolated. That colony will be suspended in MRS broth and an OD600 reading will be taken of the sample and a blank to determine a concentration. The petri dish will contain ampicillin to select for transformed bacteria only. Two millimeters of bacterial suspension will be injected into the three individual parts of the proximal colon of Tg4-42 mice and this will be done for each group. The negative control will receive LGG cells in each area of the proximal colon with a plasmid that lacks TAC1. The positive control will have no transformed proximal colon. One more added layer of complexity to help identify an optimal amount of SP (as opposed to just a location) will be the addition of the different amounts of the three SP plasmids. Each subgroup of the proximal colon will be further divided into three populations with each area receiving the one, two, or three SP gene plasmid. Thus, there will be a total of three groups of three analyzed in the proximal colon. Each of the three individual SP plasmid populations within each region of the colon will consist of n=15 mice. The experiment will begin on seven-month-old Tg4-42 mice as this is when memory decline becomes observable in this model [29].

To ensure consistent and stable expression of the TAC1 gene, syringes will be inserted into the site of inoculation before and after administration of our LGG cells. The contents on the syringe needle will then be collected, and western blots will be used to determine SP baseline levels. The HIS tag on the SP will ensure only SP produced by LGG cells are detected in the colon. This assay will rely on 'ab173828' antibodies [30] as they are conjugated to biotin

and can bind to the 6X HIS tag. This will be performed periodically to ensure that SP levels are consistent in all groups before the memory test begins. LGG cells have a short generation time (G=40mins) [31], and therefore, we suspect LGG population stabilization will only take a few hours. Once stable expression is observed, the LTM tests can begin. Both before and after the study begins Shotgun Metagenomic Sequencing will be used to assess microbial diversity using 'MetaGenome Analyzer' bioinformatics software to visualize the microbial community. This will ensure consistent environments for the LGG cells in each mouse and provide valuable insight on how the treatment influences the diversity of microbes in the gut.

To conduct the MWM test, each mouse will be placed in the same location of a pool with a platform submerged one inch under room temperature water located opposite of the mouse. A camera will track the mice's escape latency, head angle, and speed between each group and their respective subgroup. This test will be conducted two times a day for one week to ensure the mice can remember where the platform is located. Then, once a month for the next three months, the mice will be administered the MWM test to see how well they remember where the platform is. These variables (as a measure of LTM loss) from the experimental group will be compared against the two control groups. Footage from the MWM test will be analyzed using DeepLabCut software. The second LTM test that will be conducted is the BM test where each group will be placed in the same location on a circular table with 40 holes evenly spaced along the perimeter with one connected to a tunnel to escape. A bright light will be placed above the table allowing for an aversive stimulus. The mice will be placed in the center of the table and then the same variables as the MWM test will be tracked for each group and analyzed using DeepLabCut software. This experiment will be conducted one week after the MWM experiment has begun to reduce the possibility of the MWM test influencing the BM test results. The BM test will be conducted for the same number of trials and duration as the MWM. All mice from every group will also undergo a post-mortem histological assay to determine hippocampal SP concentrations. This assay will utilize 'PA5-106934' antibodies [32] conjugated to 'ab150077' [33] secondary fluorescent antibodies to allow for visualization of SP in hippocampal tissue under a light microscope. The SP produced in the gut can also be analyzed by using 'ab173828' antibodies' [30] to determine where the SP localizes to the tissue. This provides essential information about where the colon is most likely to benefit from the SP within each segment of the colon.

Results

Results can be expected within nine months as the experiment will be replicated three times with each experiment taking three months to help increase validity and accuracy of the results. While it is unclear where in the colon the proposed treatment may have its greatest therapeutic effect, it is expected that increased doses of SP will improve LTM loss. This is because AD individuals have been shown to have decreased levels of SP [34]. This reduction in SP can therefore be negated with the continuous application of SP in the gut. It is likely that larger doses (such as the 3X TAC1 gene plasmid) will delay AD progression the most. This would be in part due to the many neuroprotective effects of SP such as its ability to stimulate LTP in the hippocampus [35]. Therefore, stronger neural connections in the hippocampus may delay the impact the disease has. The effect size of each group should be large as a 1X concentration vs a 3X concentration in the probiotic will result in a large difference in the amount of SP made. This will drastically change the length of time and number of synapses the SP interact with in the gut. This effect size may eventually plateau as the colon becomes saturated with SP thus, this large effect size on reducing AD progression may be short-lived if the SP accumulates without being removed from the body. The memory test with the best treatment outcome will likely be the BM test as the MWM test uses water which is more stressful and may be more difficult for the AD model to navigate. Furthermore, the experimental group should yield a reduction in LTM loss while the positive and negative group will see a decline in LTM.

When all experimental data has been collected the following proposed statistical analysis will be performed. To determine if there is a significant difference in the measured variables of mice across the three groups a one-way Analysis of Variance (ANOVA) will be conducted. The ANOVA will be verified visually and other tests such as the Shapiro-Wilk normality test, Levene test for homoscedasticity, and Durbin-Watson test will be used for autocorrection. Results from the ANOVA test will indicate whether the means of the measured variables across the three groups are statistically different from one another. Post-hoc pairwise comparisons will be conducted using Tukey's multiple comparisons test. Results from this test will indicate which measured variables means across the three groups are statistically different from each other. Cohen's d for effect size and 95% confidence intervals will be computed to quantify the magnitude of the difference between the group means as well as determine the precision of the measurements.

Discussion

While there exists very little literature on the effect of SP in the colon on memory deficits, there is substantial literature on the effects SP has on neurons. For example, it is well understood that SP has neurogenesis-like effects [36] which indicates a potential therapeutic effect in those who could benefit from stronger/more neuron connections. SP accomplishes this by priming synapses found in the hippocampus [37]. This beneficial relationship is the basis of our proposed study. However, we are analyzing the

therapeutic effects when applied in different amounts and different areas of the colon. After the study has concluded, post-mortem histological assays will be conducted to determine if SP levels increased in the hippocampus. While this will allow us to determine if more SP is present, we will not know the mechanism by which LTM loss was reduced in the hippocampus. Since SP is implicated in improved neurogenesis, perhaps the reason for improved memory could be an increase in synaptic strength between neurons within the hippocampus. SP is also known to modulate cytokines which can reduce neuroinflammatory responses, thus this may also be the reason for reduced LTM loss [38]. These are two possible mechanisms by which the novel probiotic treatment may reduce LTM loss in AD individuals. There may exist other forms of modulation of the hippocampus upon receiving our treatment that could be assessed in future experiments. Fortunately, for a person with AD, any reduction in memory loss by any of the above mechanisms would be a welcomed result.

The concept of using probiotics to reduce AD progression has been studied in clinical settings before and there is mounting evidence that this form of treatment is viable [39]. Studies have shown that some common gut bacteria can produce neurotransmitters in the gut which can alter the GBA [40]. In the proposed study, we will take advantage of this observation; however, unlike many of the studies conducted on this topic, ours will be conducted with transformed bacteria. The use of transformed bacteria in the gut to assess LTM in AD models is a topic that has not been well researched yet. Many studies manipulated the gut microbial composition in AD individuals, as opposed to introducing transformed microbes. This is because the gut microbial environment is in a state of dysbiosis in AD individuals [41] hence, why this issue is targeted by researchers.

If LTM loss is reduced in mice, these findings will encourage other researchers to investigate the GBA. This may result in new and better therapeutic options for AD individuals in the form of probiotics. Anticipated results in this experiment will also further strengthen the poorly understood relationship between gut microbiota and cognition via the GBA. In this experiment we assess different dosages of SP and in different areas of the gut. After completion of this study it may allow for more tailored approaches to each AD individual allowing for improved treatment outcomes.

Conclusion

This research protocol seeks to understand how LTM loss in AD individuals can be reduced by utilizing a novel probiotic treatment. By sharing this protocol, it will allow for the scientific community to test this hypothesis and determine the true relationship between SP levels in the proximal colon and LTM loss. In developing this proposal, many new questions arose that will require future analysis. One future study we propose would be combinatorial studies to determine if other current cognitive enhancement treatments have a synergistic effect when combined with ours. Another future test would be molecular analysis through RNA sequencing which would determine what effects our probiotic treatment will have on gene expression in the hippocampus. This will provide useful insight into signaling mechanisms. Another future direction would be neuroinflammation analysis. We could use enzyme-linked immunosorbent assays to assess certain chemical markers (such as cytokines) to see if there is a reduction of inflammation that may be linked to AD and thus loss of memory. While the proposed study will determine which concentration and where the probiotic is most effective, future studies could determine if higher doses in earlier stages are worth the long-term exposure to the drug as opposed to increased dosage over time. This would reduce the burden of overmedication in AD individuals.

While the LTM tests utilized in this experiment analyzed a mouse's spatial memory over long periods of time (i.e., the submerged platform and escape tunnel), other metrics could be considered to evaluate treatment effectiveness. In a future study, we could analyze if the treatment results in any novel biomarkers in the hippocampus to indicate treatment effectiveness. Most importantly, future clinical studies should be conducted on people with AD. This would allow us to determine how effective this treatment is in AD individuals, and if effective, it may pave the way for a new treatment option.

List of Abbreviations Used

AD: Alzheimer's disease
LTM: long-term memory
SP: substance P
LTP: long-term potentiation
LGG: *Lactobacillus rhamnosus GG*
MRS: de Man, Rogosa and Sharpe
HIS: Histidine
MWM: Morris water maze
BM: Barnes maze
ANOVA: analysis of variance

Conflicts of Interest

The author(s) declare that they have no conflict of interests.

Ethics Approval and/or Participant Consent

The Animal Care and Ethics Committee will need to be consulted prior to experimentation to ensure the protocol meets ethical standards for the mice used.

Authors' Contributions

TP: made contributions to the design of the study, collected and analyzed data, drafted the manuscript, and gave final approval of the version to be published.

TP: contributed to study design and planning, assisted with the collection and analysis of data, and gave final approval of the version to be published.
TP: Made substantial contributions to the design of the study, the collection of data as well as interpretation and analysis of the data, revised the manuscript critically, and gave final approval of the version to be published.

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References

[1] Rajan KB, Weuve J, Barnes LL, McAninch EA, Wilson RS, Evans DA. Population estimate of people with clinical alzheimer's disease and mild cognitive impairment in the United States (2020–2060). Alzheimer's & Dementia. 2021;17(12):1966–75. https://doi.org/10.1002/alz.12362
[2] Hildebrandt H, Haldenwanger A, Eling P. False recognition correlates with amyloid-β1–42 but not with total tau in cerebrospinal fluid of patients with dementia and mild cognitive impairment. Journal of Alzheimer's Disease. 2009;16(1):157–65. https://doi.org/10.3233/jad-2009-0931
[3] Kumar A. Home - books - NCBI [Internet]. U.S. National Library of Medicine; [cited 2023 Aug 23]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499922/#:~:text=Pathophysiology,surrounded%20by%20enlarged%20axonal%20endings
[4] Medeiros R, Baglietto-Vargas D, LaFerla FM. The role of tau in alzheimer's disease and related disorders. CNS Neuroscience & Therapeutics. 2010;17(5):514–24. https://doi.org/10.1111/j.1755-5949.2010.00177.x
[5] Dhikav V, Anand K. Hippocampus in health and disease: An overview. Annals of Indian Academy of Neurology. 2012;15(4):239. https://doi.org/10.4103/0972-2327.104323
[6] Z. H. Role of preprotachykinin a (TAC1) gene-derived peptides and the neurokinin 1 (NK1) receptor in acute nocifensive behaviours and Hyperalgesia. Frontiers in Neuroscience. 2011;5. https://doi.org/10.3389/conf.fnins.2011.84.00131
[7] Chen X-Y, Du Y-F, Chen L. Neuropeptides exert neuroprotective effects in Alzheimer's disease. Frontiers in Molecular Neuroscience. 2019;11. https://doi.org/10.3389/fnmol.2018.00493
[8] Campolongo P, Ratano P, Ciotti MT, Florenzano F, Nori SL, Marolda R, et al. Systemic administration of substance P recovers beta amyloid-induced cognitive deficits in rat: Involvement of KV Potassium channels. PLoS ONE. 2013;8(11). https://doi.org/10.1371/journal.pone.0078036
[9] Chen X-Y, Du Y-F, Chen L. Neuropeptides exert neuroprotective effects in Alzheimer's disease. Frontiers in Molecular Neuroscience. 2019;11. https://doi.org/10.3389/fnmol.2018.00493
[10] Langosch JM, Kupferschmid S, Heinen M, Walden J, Herpfer I, Fiebich BL, et al. Effects of substance P and its antagonist L-733060 on long term potentiation in guinea pig hippocampal slices. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2005;29(2):315–9. https://doi.org/10.1016/j.pnpbp.2004.11.017
[11] Dong Z, Han H, Li H, Bai Y, Wang W, Tu M, et al. Long-term potentiation decay and memory loss are mediated by Ampar endocytosis. Journal of Clinical Investigation. 2014;125(1):234–47. https://doi.org/10.1172/jci77888
[12] Betthauser TJ, Bilgel M, Koscik RL, Jedynak BM, An Y, Kellett KA, et al. Multi-method investigation of factors influencing amyloid onset and impairment in three cohorts. Brain. 2022;145(11):4065–79. https://doi.org/10.1093/brain/awac213
[13] Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, Central and Enteric Nervous Systems [Internet]. U.S. National Library of Medicine; [cited 2023 Jun 14]. Available from: https://www.pubmed.ncbi.nlm.nih.gov/25830558/
[14] Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of parkinson's disease. Cell. 2016;167(6). https://doi.org/10.1016/j.cell.2016.11.018
[15] Dieterich W, Schink M, Zopf Y. Microbiota in the gastrointestinal tract. Medical Sciences. 2018;6(4):116. https://doi.org/10.3390/medsci6040116
[16] Li F, Lai M. Colorectal cancer, one entity or three. Journal of Zhejiang University SCIENCE B. 2009;10(3):219–29. https://doi.org/10.1631/jzus.b0820273
[17] Mathipa-Mdakane MG, Thantsha MS. Lacticaseibacillus rhamnosus: A suitable candidate for the construction of novel bioengineered probiotic strains for targeted Pathogen control. Foods. 2022;11(6):785. https://doi.org/10.3390/foods11060785
[18] Darbaky Y, Evrard B, Patrier S, Falenta J, Garcin S, Tridon A, et al. Oral probiotic treatment of*lactobacillus rhamnosus*LCR35®prevents visceral hypersensitivity to a colonic inflammation and an acute psychological stress. Journal of Applied Microbiology. 2016;122(1):188–200. https://doi.org/10.1111/jam.13320
[19] Patel M, Valaiyaduppu Subas S, Ghani MR, Busa V, Dardeir A, Marudhai S, et al. Role of substance P in the pathophysiology of inflammatory bowel disease and its correlation with the degree of inflammation. Cureus. 2020; https://doi.org/10.7759/cureus.11027

[20] Westerman MA, Cooper-Blacketer D, Mariash A, Kotilinek L, Kawarabayashi T, Younkin LH, et al. The relationship between AB and memory in the TG2576 mouse model of alzheimer's disease. The Journal of Neuroscience. 2002;22(5):1858–67. https://doi.org/10.1523/jneurosci.22-05-01858.2002

[21] Brandscheid C, Schuck F, Reinhardt S, Schäfer K-H, Pietrzik CU, Grimm M, et al. Altered gut microbiome composition and tryptic activity of the 5xfad alzheimer's Mouse model. Journal of Alzheimer's Disease. 2017;56(2):775–88. https://doi.org/10.3233/jad-160926

[22] Masters CL, Lewis H, Portelius E, Bouter Y. TG4-42 [Internet]. [cited 2023 Aug 23]. Available from: https://www.alzforum.org/research-models/tg4-42

[23] Bouter Y, Dietrich K, Wittnam JL, Rezaei-Ghaleh N, Pillot T, Papot-Couturier S, et al. N-truncated amyloid β (AB) 4-42 forms stable aggregates and induces acute and long-lasting behavioral deficits. Acta Neuropathologica. 2013;126(2):189–205. https://doi.org/10.1007/s00401-013-1129-2

[24] Hinteregger B, Loeffler T, Flunkert S, Neddens J, Bayer TA, Madl T, et al. Metabolic, phenotypic, and neuropathological characterization of the TG4-42 mouse model for alzheimer's disease. Journal of Alzheimer's Disease. 2021;80(3):1151–68. https://doi.org/10.3233/jad-201204

[25] Barrientos RM, Kitt MM, D'Angelo HM, Watkins LR, Rudy JW, Maier SF. Stable, long-term, spatial memory in young and aged rats achieved with a one day morris water maze training protocol. Learning & Memory. 2016;23(12):699–702. https://doi.org/10.1101/lm.043489.116

[26] Pitts M. Barnes Maze procedure for spatial learning and memory in mice. BIO-PROTOCOL. 2018;8(5). https://doi.org/10.21769/bioprotoc.2744

[27] Behavioral and Functional Neuroscience Laboratory. Modified Barnes maze test [Internet]. [cited 2023 Jun 14]. Available from: https://www.med.stanford.edu/sbfnl/services/bm/lm/bml-barnes-maze.html

[28] Heijningen S van. What is the Barnes Maze? A dry-land environment for testing learning and memory [Internet]. Noldus Information Technology; 2022 [cited 2023 Jun 14]. Available from: https://www.noldus.com/blog/what-is-the-barnes-maze#:~:text=The%20Barnes%20maze%20requires%20a,performed%20over%20%C2%B1%2012%20trials

[29] Wagner JM, Sichler ME, Schleicher EM, Franke TN, Irwin C, Löw MJ, et al. Analysis of motor function in the TG4-42 mouse model of Alzheimer's disease. Frontiers in Behavioral Neuroscience. 2019;13. https://doi.org/10.3389/fnbeh.2019.00107

[30] Biotin anti-6x his tag® antibody [HIS.H8] (AB173828) [Internet]. 2023 [cited 2023 Aug 23]. Available from: https://www.abcam.com/products/primary-antibodies/biotin-6x-his-tag-antibody-hish8-ab173828.html

[31] Hussain N, Li R, Takala TM, Tariq M, Zaidi AH, Saris PE. Generation of lactose- and protease-positive probiotic *lacticaseibacillus rhamnosus* GG by conjugation with *lactococcus lactis* NCDO 712. Applied and Environmental Microbiology. 2021;87(6). https://doi.org/10.1128/aem.02957-20

[32] Substance P polyclonal antibody [Internet]. [cited 2023 Aug 23]. Available from: https://www.thermofisher.com/antibody/product/Substance-P-Antibody-Polyclonal/PA5-106934

[33] Goat anti-rabbit IGG H&L (Alexa Fluor® 488) (AB150077) [Internet]. 2023 [cited 2023 Aug 23]. Available from: https://www.abcam.com/products/secondary-antibodies/goat-rabbit-igg-hl-alexa-fluor-488-ab150077.html?productwalltab=abreviews%7CAbreviews&productWallTab=ShowAll

[34] Severini C, Petrella C, Calissano P. Substance P and alzheimer's disease: Emerging novel roles. Current Alzheimer Research. 2016;13(9):964–72. https://doi.org/10.2174/1567205013666160401114039

[35] Dasgupta A, Baby N, Krishna K, Hakim M, Wong YP, Behnisch T, et al. Substance P induces plasticity and synaptic tagging/capture in rat hippocampal area CA2. Proceedings of the National Academy of Sciences. 2017;114(41). https://doi.org/10.1073/pnas.1711267114

[36] Zeng C, Yu Y, Shu S, Liu X, Li C. Similar effects of substance P on learning and memory function between hippocampus and striatal marginal division. Neural Regeneration Research. 2014;9(8):857. https://doi.org/10.4103/1673-5374.131603

[37] Dasgupta A, Baby N, Krishna K, Hakim M, Wong YP, Behnisch T, et al. Substance P induces plasticity and synaptic tagging/capture in rat hippocampal area CA2. Proceedings of the National Academy of Sciences. 2017;114(41). https://doi.org/10.1073/pnas.1711267114

[38] Johnson MB, Young AD, Marriott I. The therapeutic potential of targeting substance P/NK-1R interactions in inflammatory CNS disorders. Frontiers in Cellular Neuroscience. 2017;10. https://doi.org/10.3389/fncel.2016.00296

[39] Naomi R, Embong H, Othman F, Ghazi HF, Maruthey N, Bahari H. Probiotics for Alzheimer's disease: A systematic review. Nutrients. 2021;14(1):20. https://doi.org/10.3390/nu14010020

[40] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano G, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019;7(1):14. https://doi.org/10.3390/microorganisms7010014

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