

Communication

The Influence of *Lactobacillus paracasei* HII01 Supplementation on Performance in Attention (Go/No-Go) Tasks and Quinolinic Acid and 5-Hydroxyindoleacetic Acid Levels in Thai Children—A Preliminary Study

Pranom Fukngoen ^{1,2}, Bhagavathi Sundaram Sivamaruthi ^{1,3} , Sasithorn Sirilun ¹ , Ekasit Lalitsuradej ^{1,4}, Suchanat Khongtan ¹, Sartjin Peerajan ⁵, Phakkharawat Sittiprapaporn ^{4,*}  and Chaiyavat Chaiyasut ^{1,*} 

¹ Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand; pranom_fukngoen@cmu.ac.th (P.F.); sivamaruthi.b@cmu.ac.th (B.S.S.); sasithorn.s@cmu.ac.th (S.S.); ekasit_l@cmu.ac.th (E.L.); suchanat_k@cmu.ac.th (S.K.)

² Master's Degree Program in Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

³ Office of Research Administration, Chiang Mai University, Chiang Mai 50200, Thailand

⁴ Neuropsychological Research Laboratory, Department of Anti-Aging and Regenerative Science, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok 10110, Thailand

⁵ Health Innovation Institute, Chiang Mai 50200, Thailand; s.peerajan@gmail.com

* Correspondence: wichian.sit@mfu.ac.th (P.S.); chaiyavat@gmail.com (C.C.)



Citation: Fukngoen, P.; Sivamaruthi, B.S.; Sirilun, S.; Lalitsuradej, E.; Khongtan, S.; Peerajan, S.; Sittiprapaporn, P.; Chaiyasut, C. The Influence of *Lactobacillus paracasei* HII01 Supplementation on Performance in Attention (Go/No-Go) Tasks and Quinolinic Acid and 5-Hydroxyindoleacetic Acid Levels in Thai Children—A Preliminary Study. *Appl. Sci.* **2022**, *12*, 5658. <https://doi.org/10.3390/app12115658>

Academic Editors: Dorota Zielińska and Katarzyna Neffe-Skocińska

Received: 30 March 2022

Accepted: 30 May 2022

Published: 2 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Cognitive development is defined as the capacity of a child to think, reason, and use language, which are all vital to their overall growth. Attention deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder. Though several factors are associated with the incidence of ADHD, gut microbiota and gut homeostasis play critical roles in it. Gut dysbiosis and altered gut homeostasis are linked to several physical and psychological complications that affect gut–brain communication (the gut–brain axis). Probiotics, live microorganisms that confer a health benefit to the host when administered in adequate amounts, are considered therapeutic supplements that can be used to manage mental and cognitive disorders. Intervention with probiotics can improve the gut microbial ecosystem and the gut–brain axis, thereby improving cognitive function. We hypothesized that the supplementation of *Lactobacillus paracasei* HII01 might reduce the risk of the development of neuropsychiatric disorders; thus, we evaluated the efficacy of *L. paracasei* HII01 on the attention state of healthy children and the changes in representative neuroinflammatory markers. Ten healthy Thai children were supplemented with 10⁹ CFU of *L. paracasei* HII01 for 12 weeks. Go/no-go tasks were undertaken to assess changes in attention state. Alterations in brain waves were measured by electroencephalographic (EEG)/event-related potential (ERP) recordings. The levels of quinolinic acid (QA, a metabolite of tryptophan) and 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of serotonin) were determined in the urine at baseline and after 12 weeks of probiotic intervention. The levels of QA and 5-HIAA significantly decreased and increased, respectively. The QA/5-HIAA ratio also decreased significantly. Go/No-go tasks revealed that the percentages of go accuracy and go error increased and decreased significantly, respectively. EEG/ERP recordings showed that theta, alpha, and beta waves were substantially altered at the 12th week of study compared to baseline values. The results suggested that *L. paracasei* HII01 might improve the gut microbiota and oscillate the brain function, which sustained the attention state of the subjects. These preliminary findings require further detailed study to confirm the role of *L. paracasei* HII01 in the improvement in the attention of healthy children.

Keywords: *Lactobacillus paracasei* HII01; go/no-go tasks; EEG/ERP; attention; quinolinic acid; 5-hydroxyindoleacetic acid

1. Introduction

Cognitive performance, which comprises a group of functions that includes memory, general intelligence, learning, language, orientation, perception, attention, concentration, and judgment, are regarded as important factors in a child's personal and professional attainment [1,2]. In addition, cognition also refers to a child's ability to think and reason or to have executive function capacity, which plays a critical role in daily and social behaviors. The development of these abilities occurs throughout the childhood and adolescence periods and in parallel to developmental changes in the brain throughout the mentioned periods [3].

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, with a prevalence of ~5.29% [4]. The treatment procedure is complicated and is related to the psychosocial aspects of the children [5]. Genetic and environmental factors and gut microbiota play a crucial role in developing ADHD [6–9]. The pharmacological treatments for ADHD have limitations and side effects. At the same time, the efficiency of non-pharmacological treatments has not been confirmed.

Cognitive capabilities and developments are influenced by many factors, including dietary factors [10]. A strong association between nutritional intake and neurocognitive development in childhood has been reported [11]. A meta-analysis disclosed that the supplementation of free fatty acids, excluding artificial food colors, improved ADHD [12,13].

Though several factors are associated with the incidence of ADHD, gut microbiota and gut homeostasis play critical roles in it. Gut dysbiosis and altered gut homeostasis are linked to several physical and psychological complications, which affect the microbiome–gut–brain axis. Thus, the gut microbiome could be a therapeutic target for treating ADHD in children [9].

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” [14]. Probiotics are known to confer several physical and neurological health benefits in humans [15–17] and animals [18,19]. Probiotic supplementation can improve the health-related quality of life of children and adolescents with ADHD [4]. The supplementation of probiotics positively regulates and aids in retaining a healthy microbiome in humans [16,20]. *Lactobacillus rhamnosus* GG (ATCC 53103) is a well-known probiotic that influences the host's gut microbial composition and exhibits immunomodulatory properties [21]. Partty et al. [8] reported that supplementation of *L. rhamnosus* GG (ATCC 53103) in early life might reduce the risk of developing neuropsychiatric disorders.

Go/no-go tasks can be employed to assess the attention state of subjects with the support of electroencephalographic (EEG)/event-related potential (ERP) recordings, which enable the measurement of electrical brain activity.

In the go/no-go task, participants respond to either “go” or “no-go” stimuli. The commission error rate represents the “go” response made by the subject on “no-go” trials. The response inhibition is considered better when fewer errors are made [22].

ERP supports capturing the neural activity associated with sensory and cognitive processes. ERP can be measured with the help of EEGs, a procedure that measures the brain's electrical activity over time using electrodes [23].

Tryptophan is one of the essential amino acids that supports normal growth and production and maintenance of enzymes and neurotransmitters. The metabolism of tryptophan, through the kynurenine pathway (KP), produces neurotoxic compounds such as quinolinic acid (QA) [24].

Serotonin is a neurotransmitter involved in several biological functions, including cognition and memory. The metabolite 5-hydroxyindoleacetic acid (5-HIAA) is one of the metabolites of serotonin. A reduction in the level of 5-HIAA directly correlates with serotonergic activity. The clinical level of 5-HIAA has been used to assess serotonergic activity. Lower levels of 5-HIAA may be correlated with depression, aggressive or violent behavior, and obsessive-compulsive disorder [25].

Accordingly, the current study was designed to evaluate the supplementation of *Lactobacillus paracasei* HII01 on the attention state of healthy children through go/no-go tasks by measuring electrical brain activities using EEG/ERP. Moreover, changes in the representative neuroinflammatory markers (QA and 5-HIAA) were established.

2. Materials and Methods

2.1. Subjects

The study was conducted on ten Thai children. Changes in the study parameters were analyzed at baseline and after 12 weeks of intervention. Informed consent was obtained from the study subjects, and the Ethical Committee of Mae Fah Luang University approved the study protocol (code: EC21059-20). Children (male and female) between the ages of 6 and 12 years old, who understood the Thai language (i.e., they could read, write, speak, and listen to the Thai language), were screened and included. Subjects with neurological disorders, tumors, chronic enteritis, severe infectious diseases, metabolic diseases, epilepsy, food allergies, and antibiotic use (within 2 weeks before the study) were excluded.

2.2. Treatment

Lactobacillus paracasei HII01 was obtained from Organic Vita Co. Ltd., Chiang Mai, Thailand, and freeze-dried *L. paracasei* HII01 was prepared at Lactomason Co. Ltd., Jinju-si, South Korea. Dry probiotic powder (10^9 CFU per sachet) was provided to the subjects. They were advised to consume one sachet every day before lunch for 12 weeks. Data were collected at weeks 0 and 12 of the study (Figure 1).

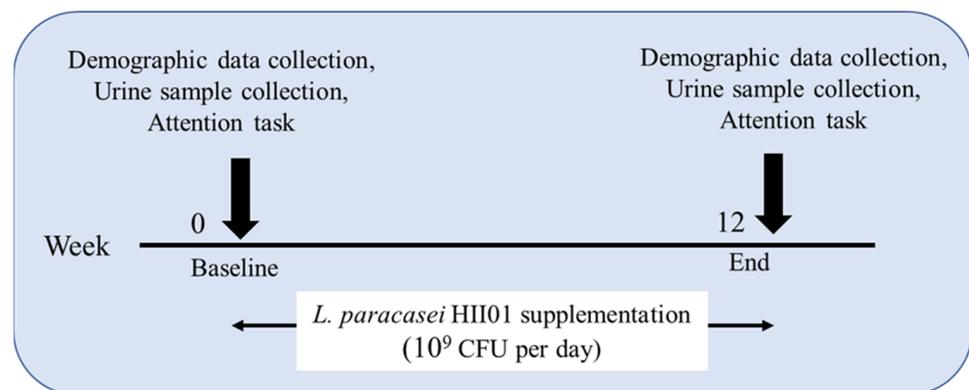


Figure 1. The timeline of the study.

2.3. Assessments of Demographic Characteristics and Neuroinflammatory Markers

The demographic characteristics of the subjects, such as age, sex, and body weight, were noted. The subjects' personal information was recorded, including physical activity, illness, and drug and food allergies.

Neuroinflammatory markers, QA and 5-HIAA, were determined in urine samples using an ELISA commercial kit (FIVEphoton Biochemicals™, San Diego, CA, USA; ImmuSmol, Bordeaux, France) as per the manufacturer's instructions. The QA/5-HIAA ratio was calculated.

2.4. Assessment of Attentional Function

The attentional function was measured by EEG/ERP analysis via the oddball visual paradigm and the cognitive battery psychological test using a computer interface. The go/no-go test was employed. It was used to monitor, update, shift, and inhibit working memory capacities. Trained research assistants assessed the experimental subjects. The accuracy and response times were recorded and expressed as a percentage of accuracy in milliseconds (ms) during the test session.

The subjects performed a modified go/no-go task [26]. Subjects were exposed to a set of fish and sharks, and they were asked to remember the fish. Subjects were required to recall them when asked to select only fish. During this task, fish and sharks that served as go and no-go signals were presented on a monitor at a distance of around 150 cm from the subjects' eyes. The fish represented the 'go' condition with 75% probability (60 stimuli), and the sharks represented the 'no-go' condition with 25% probability (20 stimuli). Reaction buttons were placed under the subjects' palms in a soundproofed and electrically protected chamber. Subjects were asked to press the response pad as quickly as possible (with their dominant hand) when they saw the fish (go stimuli) appear on the computer screen. The subjects were asked to withhold their reactions when they saw the sharks (no-go stimuli). The order of conditions was counterbalanced across subjects (Figure 2).

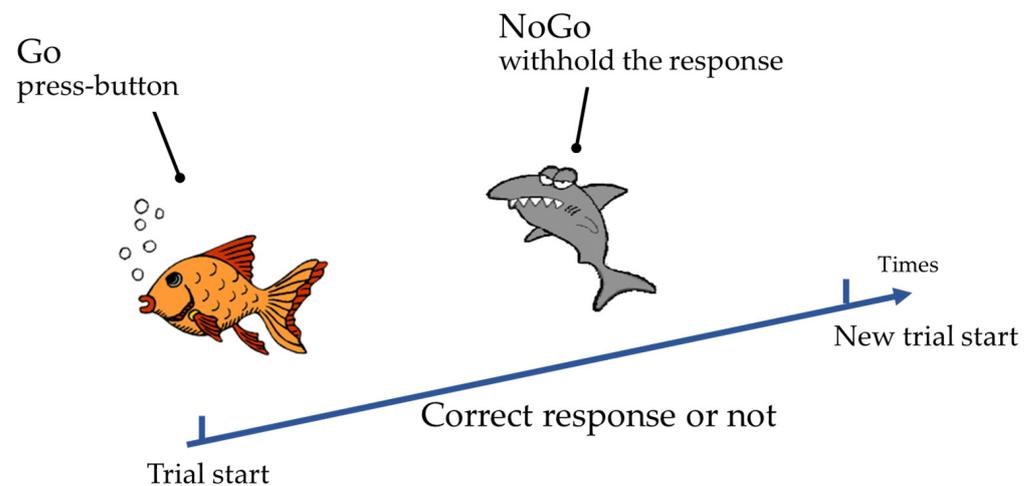


Figure 2. Schematic representation of go/no-go (fish/shark) experimental design [26].

An Electro-Cap was used for the EEG/ERP recordings (EEG, eego™, ANT Neuro, PE Hengelo, Netherlands). A 32-channel active set of electrodes was pre-mounted in an elastic Electro-Cap (Waveguard™ original EEG cap, ANT Neuro, PE Hengelo, Netherlands) according to the International 10–20 Electrode Positioning System. The Waveguard original EEG cap is lightweight, and the flexible and breathable cap fabric enables comfortable recordings. A ground electrode was inserted between the electrodes Fz and Cz. Manual reference electrodes were placed on ipsilateral mastoids (M1 and M2), and Fp1 and Fp2 electrodes were employed for ocular artifact detection. The resistance of the electrode was less than 10 kΩ.

The EEG amplified and captured the 0.05 to 100 Hz bandpass during the experiment, and data were saved for offline processing. ERPs were digitally filtered with a cut-off bandpass of 0.1–30 Hz. All artifacts were removed from the continuous EEG before extracting ERP waves. EEG was allocated into epochs ranging from –100 ms pre-stimulus to 500 ms post-stimulus. The baseline correction was applied to each epoch. The analysis excluded any voltage changes below 0.1 μV or above 70 μV from the analysis. EEG epochs with absolute amplitudes greater than 100 μV were automatically flagged and removed.

Before averaging, all channels were subjected to artifact rejection with a threshold of ±100 μV. ERP waveforms were generated to investigate the ERP components, where the target stimuli evoked reactions of frequent (go) stimuli (75% probability, 60 stimuli). The infrequent (no-go) stimuli were non-target conditions presented randomly with a probability of 25% and 20 stimuli, and this was named the oddball paradigm.

The interstimulus interval was 1000 ms. The amplitude (μV) and latency (ms) of the ERP signals were measured. Two blocks of cognitive function tests were provided to the subjects. Subjects were informed to pay attention to the stimuli of each block of the cognitive test and respond to each stimulus by pressing the response button in front of them. The total recording time was 5 min for each block of the cognitive test. The analysis was performed using ASATM 4.0 analysis software (ANT Neuro, PE Hengelo, The Netherlands). The ASATM is a highly flexible EEG/ERP and the MEG analysis software package provides source reconstruction, signal analysis, and MRI processing tools. The positive peak between 250 and 400 ms was defined as P300 [27]. Both latencies and amplitudes of brainwaves were recorded and analyzed.

The average amplitudes from 32 active channel electrodes were defined as a moment of the global field power (GFP) with the peak of an epoch related to stable scalp-potential topography. sLORETA was applied to estimate the current source density distribution in the brain, which contributed to the electrical scalp field. sLORETA computed the smoothest of all possible source configurations throughout the brain volume by minimizing the total squared Laplacian of the source strengths [28].

The GFP peak metric was used to assess the brain's electric strength (hilliness), and the electric field map is independent of its spatial layout. A steep potential map would have a higher GFP peak than a flat potential map. The GFP described above was self-contained [28,29]. The spatial standard deviation of global field power quantified the activity at each time point in the field, resulting in a reference-independent descriptor of the potential field. The occurrence times of the GFP maxima were used to determine the latencies of evoked potential components. Over time, GFP is complementary [30].

Visual stimuli elicited the principles and practical applications of the GFP calculation, component latency determination, global dissimilarity of potential field distributions, and a topographical temporal segmentation process presented with multichannel data. This was done by averaging all scalp channels' evoked potentials (EPs), excluding electrooculographic channels. Subjects' mean GFP peak amplitudes were computed. The subjects' grand mean GFP peak amplitudes were also computed [28–31].

2.5. Statistical Analyses

Demographic data were represented as mean \pm standard error (SE). The other parameters were analyzed using the paired *t*-test or Wilcoxon matched-pairs, signed-rank test using STATA version 15.1 (StataCorp, College Station, TX, USA).

3. Results

Six male and four female subjects were involved in the study. The demographic information (age, sex, and body weight) of the participants was provided (Table 1). All the subjects ($n = 10$) completed the study without any side effects or hindrances.

Table 1. The subjects' demographic data ($n = 10$, mean \pm SE).

Parameters	Baseline	12 Weeks	<i>p</i> -Value *
Age (years)		9.50 \pm 0.50	
Male, <i>n</i> (%)		6 (60)	
Female, <i>n</i> (%)		4 (40)	
Bodyweight (kg)	55.62 \pm 5.18	55.68 \pm 5.00	0.885

* Calculated by paired *t*-test.

The baseline value of QA was 26.73 ± 1.42 ng/mL, whereas the 12th week QA level was 19.64 ± 1.08 ng/mL. A significant reduction ($p = 0.004$) was observed in the QA level. The baseline value of 5-HIAA was 2.91 ± 0.44 mg/L, whereas the 12th week 5-HIAA level was 7.28 ± 1.19 mg/L. A significant increase level ($p = 0.005$) was observed in the 5-HIAA level. The ratios of QA/5-HIAA were 0.0109 ± 0.001 and 0.0043 ± 0.001 at baseline and at the 12th week of the study, respectively. The change in QA/5-HIAA ratio was significant ($p = 0.005$) (Table 2).

The go accuracy was $85.58 \pm 2.46\%$ at baseline, whereas it was $97.92 \pm 0.97\%$ at the end of the study (12th week). The go error rates were 14.42 ± 2.46 and $2.08 \pm 0.97\%$ at baseline and the 12th week, respectively. The go accuracy level increased, and the go error level decreased by the end of the study. The changes were significant in both go accuracy ($p = 0.001$) and go error ($p = 0.006$) (Table 3).

Table 2. Changes in QA, 5-HIAA, and QA/5-HIAA ratio during the study ($n = 10$, mean \pm SE).

Parameters	Baseline	12 Weeks	<i>p</i> -Value
QA (ng/mL)	26.73 ± 1.42	19.64 ± 1.08	0.004 ^{a*}
5-HIAA (mg/L)	2.91 ± 0.44	7.28 ± 1.19	0.005 ^{b*}
QA/5-HIAA ratio	0.0109 ± 0.001	0.0043 ± 0.001	0.005 ^{b*}

* Statistically significant change ($p < 0.05$). ^a calculated by paired *t*-test. ^b calculated by Wilcoxon matched-pairs, signed-rank test.

Table 3. Attention test (go/no-go task) responses ($n = 10$, mean \pm SE).

Parameters	Baseline	12 Weeks	<i>p</i> -Value
Go accuracy (%)	85.58 ± 2.46	97.92 ± 0.97	0.001 ^{a*}
Go error (%)	14.42 ± 2.46	2.08 ± 0.97	0.006 ^{b*}
No-go accuracy (%)	88.25 ± 3.36	90.75 ± 3.23	0.389 ^a
No-go error (%)	11.75 ± 3.38	9.25 ± 3.23	0.324 ^b
Go response time (ms)	599.19 ± 32.57	610.61 ± 32.99	0.649 ^a

* Statistically significant change ($p < 0.05$). ^a calculated by paired *t*-test. ^b calculated by Wilcoxon matched-pairs signed-rank test.

The no-go accuracy was $88.25 \pm 3.36\%$ at baseline, whereas it was $90.75 \pm 3.23\%$ at the end of the study (12th week). The no-go error rates were 11.75 ± 3.38 and $9.25 \pm 3.23\%$ at baseline and the 12th week, respectively. The no-go accuracy level increased, and the no-go error level decreased by the end of the study, but the changes were not significant. The go response time increased (from 599.19 ± 32.57 to 610.61 ± 32.99 ms), but it was not statistically significant ($p = 0.649$) (Table 3).

EEG/ERP recordings showed that brain wave changes occurred in the experimental subjects. Beta wave activity (from 4.73 ± 0.78 to 2.85 ± 0.32 μ V, $p = 0.005$) was reduced after 12 weeks of probiotic supplementation. The activity of theta (from 2.95 ± 0.46 to 5.66 ± 0.63 μ V, $p = 0.002$) and alpha (from 2.33 ± 0.51 to 3.63 ± 0.54 μ V, $p = 0.005$) waves increased significantly after 12 weeks of probiotic supplementation (Figure 3).

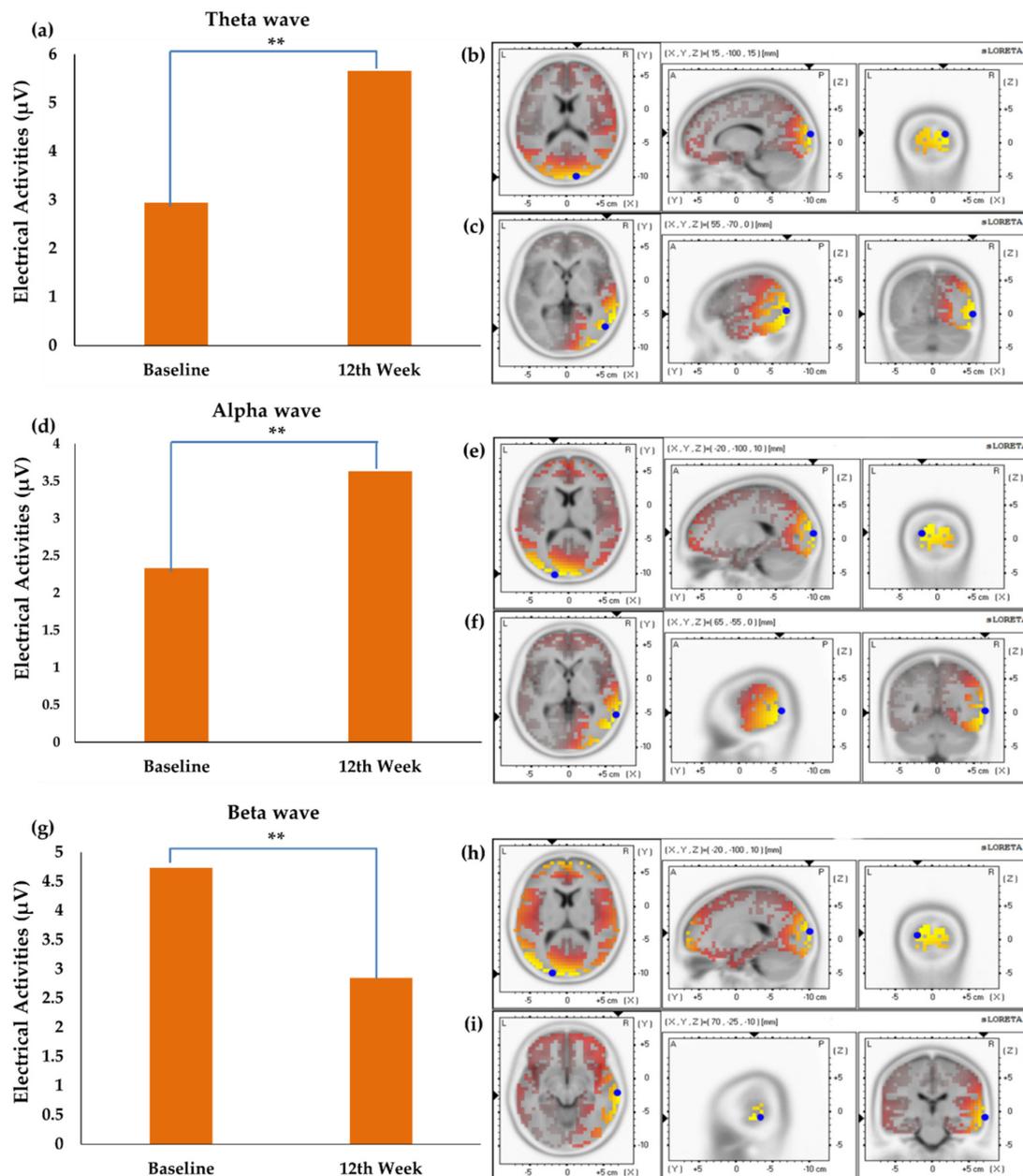


Figure 3. Change in brain oscillation and topography localization after the 12th week of *L. paracasei* HIII01 administration. (a) Modulation of theta oscillation; $p = 0.002$. (b) Theta wave activity at baseline (Brodmann area 18; $X = 15$, $Y = -100$, $Z = 15$; MNI coords; $2.95 \mu\text{V}$) in occipital gyrus of right-hemisphere. (c) Theta wave activity at the 12th week (Brodmann area 37; $X = 55$, $Y = -70$, $Z = 0$; MNI coords; $5.66 \mu\text{V}$) in middle temporal gyrus of right-hemisphere. (d) Modulation of alpha oscillation; $p = 0.005$. (e) Alpha wave activity at baseline (Brodmann area 19; $X = -20$, $Y = -100$, $Z = 10$; MNI coords; $2.33 \mu\text{V}$) in middle occipital gyrus of right-hemisphere. (f) Alpha wave activity at the 12th week (Brodmann area 37; $X = 65$, $Y = -55$, $Z = 0$; MNI coords; $3.63 \mu\text{V}$) in middle temporal gyrus of right-hemisphere. (g) Modulation of beta oscillation; $p = 0.005$. (h) Beta wave activity at baseline (Brodmann area 19; $X = -20$, $Y = -100$, $Z = 10$; MNI coords; $4.73 \mu\text{V}$) in middle occipital gyrus of right-hemisphere. (i) Beta wave activity at the 12th week (Brodmann area 37; $X = 70$, $Y = -25$, $Z = -10$; MNI coords; $2.85 \mu\text{V}$) in middle temporal gyrus of right-hemisphere. Yellow color indicates local maxima of increased electrical activity in right hemisphere. Blue dot marks center of significantly increased electrical activities. ** significant difference $p < 0.005$.

4. Discussion

In the present study, a significant level of reduction was observed in QA, while the 5-HIAA level increased significantly after the 12 weeks of *L. paracasei* HII01 supplementation (Table 2).

It has been proven that lower serum levels of tryptophan, kynurenic acid, xanthurenic acid, and 3-hydroxyanthranilic acid are associated with ADHD adults [32]. Lower levels of 3-hydroxykynurenine in ADHD children have been related to delayed brain maturation [33]. Evangelisti et al. [34] reported changes in serum levels of kynurenine metabolites in ADHD subjects. Serum levels of anthranilic, kynurenic, and xanthurenic acid were lower in ADHD children compared to healthy controls; however, kynurenine and tryptophan concentrations increased. There were no changes observed in QA concentration. The statistical analysis suggested that lower anthranilic acid and high tryptophan were associated with ADHD, which might be a biomarker for ADHD [34]. It is known that the etiology of neurological complications associated with inflammation, oxidative stress, and excitotoxicity might alter the metabolites of KP. Studies suggest that neurological disease and disorder complications could activate KP inappropriately, leading to increased QA levels [35]. In the present study, levels of QA in the urine were significantly reduced after the 12-week supplementation of *L. paracasei* HII01 in healthy children (from 26.73 ± 1.42 to 19.64 ± 1.08 ng/mL) (Table 2). Therefore, we can hypothesize that probiotic supplementation protects the host by hindering the inappropriate activation of KP. However, further studies are needed to confirm these statements.

Reduced serotonergic activity in the brain is related to the susceptibility to depression and mania. Reduction in serotonergic activity is directly correlated with 5-HIAA concentration [36]. Lower levels of 5-HIAA may be correlated with depression, aggressive or violent behavior, and obsessive-compulsive disorder [25]. Reduced cerebrospinal fluid levels of 5-HIAA have been observed in Alzheimer's patients [37]. Moreover, tryptophan metabolites such as serotonin correlate with cognition, and neurological and psychiatric disorders have been related to reduced levels of 5-HIAA [37]. The current study results revealed that the supplementation of *L. paracasei* HII01 significantly improved urine 5-HIAA levels in children (Table 2).

An increased QA/5-HIAA ratio indicates inflammation and immune stimulation. The host-microbiota regulates serotonin synthesis in intestinal enterochromaffin cells and serotonergic signaling. Serotonin influences mucosal inflammation, immune responses, and brain activity in the host. Host microbiota-mediated serotonergic signaling is involved in intestinal diseases and disorders [38]. Accordingly, *L. paracasei* HII01 supplementation reduced the QA/5-HIAA ratio (Table 2) in healthy children, possibly due to improved gut microbiota after the probiotic supplementation.

We observed significant changes in both go accuracy and go error. However, the changes in both no-go accuracy and no-go error levels were not significant. The go response time increased, but this was not statistically significant. Beta wave activity was reduced, while theta and alpha wave activity increased significantly after 12 weeks of *L. paracasei* HII01 supplementation (Figure 3).

There was no concrete evidence for the influence of probiotic intervention on go/no-go task responses and EEG/ERP. Liao et al. reported that the supplementation of long-chain polyunsaturated fatty acids significantly improved behavior and ERP while children performed a go/no-go task [26].

Similarly, the n-back/no-go combination paradigm and ERP were used to study working memory and response inhibition in ADHD subjects. The n-back/no-go combination paradigm indicated that ADHD patients have problems with their working memory and reaction inhibition, higher omission errors, [slower/faster] reaction times, and decreased n-back and no-go-P3 amplitudes [39].

Michalowski et al. evaluated the influence of motivational context on academic procrastinators using ERP and go/no-go tasks. Increased post-error slowing, reduced P300, and related negativity amplitudes were observed in high procrastination subjects,

showing impaired attention and error-related processing in this group. The results revealed increased post-error slowing along with reduced P300 and error-related negativity (ERN) amplitudes in high (vs. low) procrastination participants, effects that indicated impaired attention and error-related processing in this group. Subsequently, increased reaction time variability was observed while they performed the more difficult task, possibly due to executive attention deficits. The study suggested that procrastinators might have difficulties in executive functioning, especially in extremely challenging situations [40].

The present study showed that *L. paracasei* HII01 supplementation for 12 weeks improved go responses significantly in children (Table 3).

The modulation of gut homeostasis alters brain communication with the brain. Several channels (cholinergic, dopaminergic, GABAergic, serotonergic, and norepinephrine pathways) have been revealed by which type of gut–brain communication may occur. The modulation of these routes alters the behavior of neuronal electrolytes (Ca^{2+} , Mg^{2+} , and K^{+}) in the astrocyte, resulting in the modification of brain oscillation [41,42]. The results of the current study indicated that probiotic administration promotes brain activity. Theta oscillations are slow brain waves, while the rest are fast. The rising of theta and alpha oscillation indicated the relaxation state of the host, which was observed in the probiotic-supplemented subjects (Figure 3). Even though alpha was identified as the fast brain wave, it represents relaxation with a better speed of cognitive performance [43], indicating that the probiotic-supplemented subjects were calm, and their cognitive function was improved. Additionally, an elevated theta wave indicated the subject's sustained attention associated with better accuracy in the go task and error reduction results (Table 3).

We reported on the efficiency of *L. paracasei* HII01 in improving the attention state of healthy subjects. The supplementation of *L. paracasei* HII01 for 12 weeks improved the attention state of the subjects. However, the present study had several limitations, including using a single probiotic strain, fewer experimental subjects, limited assessed parameters, no microbiota analysis, and no follow-up study. We could not correlate the results of the present study with the mental health state of the general population of Thai subjects. Consequently, the results must be confirmed with extended studies, which may aid in developing a probiotic-based adjuvant intervention to improve cognitive function in children.

5. Conclusions

The results revealed that *L. paracasei* HII01 supplementation promotes brain activity in children. An elevated theta wave indicated the subject's sustained attention, which was associated with better accuracy in the go task and an error reduction in the results. Theta and alpha oscillations revealed that the probiotic-supplemented subjects were more relaxed and calmer than at the baseline. Collectively, *L. paracasei* HII01 supplementation positively affected brain waves and changed the levels of neuroinflammatory markers in children. With further scientific confirmation, *L. paracasei* HII01 could be used as a therapeutic supplement to promote cognitive development in children.

Author Contributions: Conceptualization, C.C., P.S., S.S. and B.S.S.; methodology, P.S. and C.C.; software, P.S.; validation, P.S., C.C. and S.S.; formal analysis, P.F., S.K., P.S. and E.L.; investigation, P.F., S.K. and P.S.; resources, C.C.; data curation, P.F. and S.K.; writing—original draft preparation, B.S.S., P.S., C.C. and E.L.; writing—review and editing, B.S.S., C.C. and P.S.; visualization, P.S.; supervision, C.C., S.S. and P.S.; project administration, C.C. and P.S.; funding acquisition, C.C. and S.P. All authors have read and agreed to the published version of the manuscript.

Funding: Pranom Fukngoen was funded by the Research and Researchers for Industries Program (RRI) under the Thailand Research Fund (TRF) (grant number MSD61I0020). This research was supported by Chiangmai University.

Institutional Review Board Statement: The study was conducted after approval from the Ethical Committee, Mae Fah Luang University, Thailand (Code: EC21059-20).

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study.

Data Availability Statement: The data presented in this study are available within the article.

Acknowledgments: The authors gratefully acknowledge Chiang Mai University and Mae Fah Luang University, Thailand, for their support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Stevens, C.; Bavelier, D. The role of selective attention on academic foundations: A cognitive neuroscience perspective. *Dev. Cogn. Neurosci.* **2012**, *2*, S30–S48. [[CrossRef](#)] [[PubMed](#)]
2. Jacob, R.; Parkinson, J. The potential for school-based interventions that target executive function to improve academic achievement. *Rev. Educ. Res.* **2015**, *85*, 512–552. [[CrossRef](#)]
3. Weintraub, S.; Dikmen, S.S.; Heaton, R.K.; Tulsky, D.S.; Zelazo, P.D.; Bauer, P.J.; Carlozzi, N.E.; Slotkin, J.; Blitz, D.; Wallner-Allen, K.; et al. Cognition assessment using the NIH Toolbox. *Neurology* **2013**, *80*, S54–S64. [[CrossRef](#)] [[PubMed](#)]
4. Kumperscak, H.G.; Gricar, A.; Ülen, I.; Micetic-Turk, D. A pilot randomized control trial with the probiotic strain *Lactobacillus rhamnosus* GG (LGG) in ADHD: Children and adolescents report better health-related quality of life. *Front. Psychiatry* **2020**, *11*, 181. [[CrossRef](#)] [[PubMed](#)]
5. Taylor, E.; Döpfner, M.; Sergeant, J.; Asherson, P.; Banaschewski, T.; Buitelaar, J.; Coghill, D.; Danckaerts, M.; Rothenberger, A.; Sonuga-Barke, E.; et al. European clinical guidelines for hyperkinetic disorder—first upgrade. *Eur. Child Adolesc. Psychiatry* **2004**, *13*, 17–30. [[CrossRef](#)]
6. Van Loo, K.M.; Martens, G.J. Genetic and environmental factors in complex neurodevelopmental disorders. *Curr. Genom.* **2007**, *8*, 429–444. [[CrossRef](#)]
7. Cenit, M.C.; Nuevo, I.C.; Codoñer-Franch, P.; Dinan, T.G.; Sanz, Y. Gut microbiota and attention deficit hyperactivity disorder: New perspectives for a challenging condition. *Eur. Child Adolesc. Psychiatry* **2017**, *26*, 1081–1092. [[CrossRef](#)]
8. Pärtty, A.; Kalliomäki, M.; Wacklin, P.; Salminen, S.; Isolauri, E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. *Pediatr. Res.* **2015**, *77*, 823–828. [[CrossRef](#)]
9. Checa-Ros, A.; Jeréz-Calero, A.; Molina-Carballo, A.; Campoy, C.; Muñoz-Hoyos, A. Current evidence on the role of the gut microbiome in ADHD pathophysiology and therapeutic implications. *Nutrients* **2021**, *13*, 249. [[CrossRef](#)]
10. Benton, D. The influence of children’s diet on their cognition and behavior. *Eur. J. Nutr.* **2008**, *47*, 25–37. [[CrossRef](#)]
11. Nyaradi, A.; Li, J.; Hickling, S.; Foster, J.; Oddy, W.H. The role of nutrition in children’s neurocognitive development, from pregnancy through childhood. *Front. Hum. Neurosci.* **2013**, *7*, 97. [[CrossRef](#)] [[PubMed](#)]
12. Sonuga-Barke, E.J.; Brandeis, D.; Cortese, S.; Daley, D.; Ferrin, M.; Holtmann, M.; Stevenson, J.; Danckaerts, M.; van der Oord, S.; Döpfner, M.; et al. Nonpharmacological interventions for ADHD: Systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *Am. J. Psychiatry* **2013**, *170*, 275–289. [[CrossRef](#)] [[PubMed](#)]
13. Rosi, E.; Grazioli, S.; Villa, F.M.; Mauri, M.; Gazzola, E.; Pozzi, M.; Molteni, M.; Nobile, M. Use of non-pharmacological supplementations in children and adolescents with Attention Deficit/Hyperactivity Disorder: A critical review. *Nutrients* **2020**, *12*, 1573. [[CrossRef](#)]
14. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [[CrossRef](#)] [[PubMed](#)]
15. Wang, L.J.; Yang, C.Y.; Kuo, H.C.; Chou, W.J.; Tsai, C.S.; Lee, S.Y. Effect of *Bifidobacterium bifidum* on clinical characteristics and gut microbiota in Attention-Deficit/Hyperactivity Disorder. *J. Pers. Med.* **2022**, *12*, 227. [[CrossRef](#)]
16. Sivamaruthi, B.S.; Suganthi, N.; Kesika, P.; Chaiyasut, C. The role of microbiome, dietary supplements, and probiotics in autism spectrum disorder. *Int. J. Environ. Res. Public Health* **2020**, *17*, 2647. [[CrossRef](#)]
17. Kesika, P.; Sivamaruthi, B.S.; Chaiyasut, C. Do probiotics improve the health status of individuals with diabetes mellitus? A review on outcomes of clinical trials. *BioMed Res. Int.* **2019**, *2019*, 1531567. [[CrossRef](#)]
18. Anee, I.J.; Alam, S.; Begum, R.A.; Shahjahan, R.M.; Khandaker, A.M. The role of probiotics on animal health and nutrition. *JoBAZ* **2021**, *82*, 52. [[CrossRef](#)]
19. Sivamaruthi, B.S.; Kesika, P.; Chaiyasut, C. Influence of probiotic supplementation on health status of the dogs: A review. *Appl. Sci.* **2021**, *11*, 11384. [[CrossRef](#)]
20. Kalenik, A.; Kardaś, K.; Rahnema, A.; Sirojć, K.; Wolańczyk, T. Gut microbiota and probiotic therapy in ADHD: A review of current knowledge. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2021**, *110*, 110277. [[CrossRef](#)]
21. Segers, M.E.; Lebeer, S. Towards a better understanding of *Lactobacillus rhamnosus* GG-host interactions. *Microb. Cell Fact.* **2014**, *13*, S7. [[CrossRef](#)]
22. Gomez, P.; Ratcliff, R.; Perea, M. A model of the go/no-go task. *J. Exp. Psychol. Gen.* **2007**, *136*, 389–413. [[CrossRef](#)] [[PubMed](#)]
23. Sur, S.; Sinha, V.K. Event-related potential: An overview. *Ind. Psychiatry J.* **2009**, *18*, 70–73. [[CrossRef](#)] [[PubMed](#)]

24. Stone, T.W.; Forrest, C.M.; Darlington, L.G. Kynurenines and brain development. In *Targeting the Broadly Pathogenic Kynurenine Pathway*, 1st ed.; Sandeep, M., Ed.; Springer: Cham, Switzerland, 2015; pp. 45–61.
25. Spreux-Varoquaux, O.; Alvarez, J.C.; Berlin, I.; Batista, G.; Despierre, P.G.; Gilton, A.; Cremniter, D. Differential abnormalities in plasma 5-HIAA and platelet serotonin concentrations in violent suicide attempters: Relationships with impulsivity and depression. *Life Sci.* **2001**, *69*, 647–657. [[CrossRef](#)]
26. Liao, K.; McCandliss, B.D.; Carlson, S.E.; Colombo, J.; Shaddy, D.J.; Kerling, E.H.; Lepping, R.J.; Sittiprapaporn, W.; Cheatham, C.L.; Gustafson, K.M. Event-related potential differences in children supplemented with long-chain polyunsaturated fatty acids during infancy. *Dev. Sci.* **2017**, *20*, e12455. [[CrossRef](#)]
27. Haag, M. Essential fatty acids and the brain. *Can. J. Psychiatry* **2003**, *48*, 195–203. [[CrossRef](#)]
28. Lehmann, D.; Skrandies, W. Principles of spatial analysis. In *Methods of Analysis of Brain Electrical and Magnetic Signals (Handbook of Electroencephalography and Clinical Neurophysiology, Revised Series)*, 1st ed.; Gevins, A.S., Remond, A., Eds.; Elsevier Science Ltd.: Amsterdam, The Netherlands, 1987; Volume 1, pp. 309–354.
29. Pascual-Marqui, R.D.; Michel, C.M.; Lehmann, D. Segmentation of brain electrical activity into microstates: Model estimation and validation. *IEEE Trans. Biomed. Eng.* **1995**, *42*, 658–665. [[CrossRef](#)]
30. Skrandies, W. Global field power and topographic similarity. *Brain Topogr.* **1990**, *3*, 137–141. [[CrossRef](#)]
31. Bann, S.A.; Herdman, A.T. Event Related Potentials Reveal Early Phonological and Orthographic Processing of Single Letters in Letter-Detection and Letter-Rhyme Paradigms. *Front. Hum. Neurosci.* **2016**, *10*, 176. [[CrossRef](#)]
32. Aarsland, T.I.; Landaas, E.T.; Hegvik, T.A.; Ulvik, A.; Halmoy, A.; Ueland, P.M.; Haavik, J. Serum concentrations of kynurenines in adult patients with attention deficit hyperactivity disorder (ADHD): A case-control study. *Behav. Brain Funct.* **2015**, *11*, 36. [[CrossRef](#)]
33. Oades, R.D.; Dauvermann, M.R.; Schimmelmann, B.G.; Schwarz, M.J.; Myint, A.M. Attention-deficit hyperactivity disorder (ADHD) and glial integrity: S100B, cytokines and kynurenine metabolism—effects of medication. *Behav. Brain Funct.* **2010**, *6*, 29. [[CrossRef](#)] [[PubMed](#)]
34. Evangelisti, M.; De Rossi, P.; Rabasco, J.; Donfrancesco, R.; Lionetto, L.; Capi, M.; Sani, G.; Simmaco, M.; Nicoletti, F.; Villa, M.P. Changes in serum levels of kynurenine metabolites in paediatric patients affected by ADHD. *Eur. Child Adolesc. Psychiatry* **2017**, *26*, 1433–1441. [[CrossRef](#)] [[PubMed](#)]
35. Lugo-Huitrón, R.; Ugalde Muñiz, P.; Pineda, B.; Pedraza-Chaverri, J.; Ríos, C.; Pérez-de la Cruz, V. Quinolinic acid: An endogenous neurotoxin with multiple targets. *Oxidative Med. Cell. Longev.* **2013**, *2013*, 104024. [[CrossRef](#)]
36. Jayamohan, H.; Kumar, M.K.M.; Aneesh, T.P. 5-HIAA as a Potential Biological Marker for Neurological and Psychiatric Disorders. *Adv. Pharm. Bull.* **2019**, *9*, 374–381. [[CrossRef](#)] [[PubMed](#)]
37. Morimoto, S.; Takao, M.; Hatsuta, H.; Nishina, Y.; Komiya, T.; Sengoku, R.; Nakano, Y.; Uchino, A.; Sumikura, H.; Saito, Y.; et al. Homovanillic acid and 5-hydroxyindole acetic acid as biomarkers for dementia with Lewy bodies and coincident Alzheimer’s disease: An autopsy-confirmed study. *PLoS ONE* **2017**, *12*, e0171524. [[CrossRef](#)] [[PubMed](#)]
38. Mishima, Y.; Ishihara, S. Enteric microbiota-mediated serotonergic signaling in pathogenesis of irritable bowel syndrome. *Int. J. Mol. Sci.* **2021**, *22*, 10235. [[CrossRef](#)] [[PubMed](#)]
39. Breitling-Ziegler, C.; Tegelbeckers, J.; Flechtner, H.H.; Krauel, K. Economical Assessment of Working Memory and Response Inhibition in ADHD Using a Combined *n*-back/Nogo Paradigm: An ERP Study. *Front. Hum. Neurosci.* **2020**, *14*, 322. [[CrossRef](#)]
40. Michałowski, J.M.; Wiwatowska, E.; Weymar, M. Brain potentials reveal reduced attention and error-processing during a monetary Go/No-Go task in procrastination. *Sci. Rep.* **2020**, *10*, 19678. [[CrossRef](#)]
41. Thiele, A.; Bellgrove, M.A. Neuromodulation of Attention. *Neuron* **2018**, *97*, 769–785. [[CrossRef](#)]
42. Buskila, Y.; Bellot-Saez, A.; Morley, J.W. Generating brain waves, the power of astrocytes. *Front. Neurosci.* **2019**, *13*, 1125. [[CrossRef](#)]
43. Wilckens, K.A.; Ferrarelli, F.; Walker, M.P.; Buysse, D.J. Slow-wave activity enhancement to improve cognition. *Trends Neurosci.* **2018**, *41*, 470–482. [[CrossRef](#)] [[PubMed](#)]