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Role of Probiotic Microorganisms in the Brain Plasticity Development

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Synaptic Plasticity

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ABSTRACT

Probiotics are defined as beneficial microorganisms that are responsible for the maintenance of homeostasis mechanisms within the host system, especially in humans. Other than homeostasis, it is also used to improve a host system's cognition, immune functions, and antioxidant levels. Over the past decades, probiotic microorganisms have been used most commonly as traditional fermented foods in our country and some parts of southeast asia. These fermented food products majorly consist of *Lactobacillus* species, including *Lactobacillus acidophilus*, *L. fermentum*, and *L. plantarum*. The present study explored the potential role of three different lactobacillus strains (*L. acidophilus*, *L. fermentum*, and *L. Plantarum*) in forming brain plasticity changes (BPC) with the help of a cue-based learning paradigm (CBLP). Two staged behavioral studies were conducted for all behavioral analysis groups (BAG) before (without probiotic infusions - WiPI) and after probiotic infusions (with probiotic infusions - WPI) in RBLP. Behavioral responses of the WiPI & WPI phases showed the effect of a stress-free habituated environment in developing BPC and strengthening of BPC by oral infusions of probiotic microorganisms (PM). WiPI and WPI behavioral analysis were used in this study to validate BPC in a laboratory-controlled environment. Infusion of probiotic microorganisms through oral passage may have a more significant impact on the synthesis, production, and transmission of neurotransmitter precursor compounds (NPC) from the gut to the central nervous system (CNS) through the blood-brain barrier (BBB). Increased transmission of the NPC strengthens the formed plasticity changes, which results in the formation of cognitive memory functions. Thus, the present study proved that probiotic microorganisms may play a major role in cognition development through the BPC.

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1 Introduction

Synaptic plasticity is a unique adaptive feature of our central nervous system that uses activity-dependent mechanisms to restructure/alter the strength of neuronal connections based on stimulus exposure (Chaudhury et al. 2016; Kim et al. 2018; Abraham et al. 2019; Appelbaum et al. 2023; Mukilan et al. 2024a). It was already reported that synaptic plasticity plays an unavoidable role in incorporating beneficial/harmful experiences in the form of long-lasting information in different brain regions during the formation of learning and memory (LM) (Ramirez and Arbuckle 2016; Appelbaum et al. 2023; Mukilan 2023). This formed LM follows two distinct molecular mechanisms for the acquaintance and retrieval of learned information in the brain. These two molecular mechanisms result in the formation of short-term (ST) memory and long-term (LT) memory. ST and LT memory use existing proteins and RNA-dependent-protein synthesis mechanisms to form memory. Formed ST and LT memory will last for 2-6 hours (ST) until the end of life (LT). However, the time needed to form LTM is high compared to STM (Norris 2017; Abraham et al. 2019; Ashok et al. 2019; Evans et al. 2021; Luis and Ryan 2022; Mukilan et al. 2024b). For memory formation, LTM uses specific neuronal signaling pathways like cAMP response element binding protein (CREB)–mediated neuronal signaling pathway (MNSP). This CREB-MNSP is initiated by releasing specific neurotransmitters like 5-HT from the presynaptic neuron into the synaptic cleft (SC). In the SC, released neurotransmitter binds to the specific postsynaptic neuronal receptors (PNR)(Ganesh et al. 2010; Ganesh et al. 2012; Mukilan et al. 2015; Ortega-Martínez 2015; Rajan 2021). Binding neurotransmitters with the PNR results in calcium influx (CI) induction. CI influx later activates adenyl cyclase (AC), cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), and enzyme-regulated kinase – 1 (ERK – 1). Upregulation of AC, cAMP, PKA, and ERK – 1 later results in the activation and phosphorylation of CREB, which induces immediate-early genes (IEG) and postsynaptic density (PSD) protein expressions. Reliable expressions of these neuronal signaling molecules are involved in the formation of LT memory in the different brain regions (Peng et al. 2010; Mukilan et al. 2018a, 2018b; Bai and Suzuki 2020; Lin et al. 2021; Mukilan 2022; Mukilan 2023).

Reported research findings showed that oral/gut-beneficial microflora are responsible for the maintenance of the brain homeostasis mechanisms of a host (Appleton 2018; Gentile and Weir 2018; Suganya and Koo 2020). In a healthy state, some oral/gut bacterial strains are unavoidable in synthesizing and producing neurotransmitter precursor compounds (NPCs) in the gut. Produced NPCs are transported from the gut to the CNS through the BBB (Misiak et al. 2020; Mukilan 2023). Transmitted NPCs are involved in the production of a wide range of neurotransmitters [serotonin (5-HT), γ-aminobutyric acid (GABA), dopamine (DA), and noradrenaline (NA)] from the presynaptic neurons for the induction of neuronal signaling pathways (NSP) (Chen et al. 2017; O'Donnell et al. 2020; Sengupta 2020; Dicks 2022). Induction of NSP forms the cognitive memory in the olfactory bulb, hippocampus, and amygdala. Thus, the formed cognitive memory inter-relates the oral-gut-brain axis with the CNS. It also proved that oral/gut-beneficial flora are important in forming cognitive functions (Mukilan et al. 2018a; Salami and Soheili 2022; Mukilan 2023). In normal conditions, existing beneficial flora is affected by various conditions like food habits, alcohol consumption, changes in circadian rhythm, alcohol consumption, diet, smoking, and other environmental conditions. To overcome the depletion of beneficial flora, nowadays's varying range of probiotic microorganisms are taken along with the diet to maintain oral/gut probiotic health (Hillemacher et al. 2018; Savin et al. 2018; Yang et al. 2020; Kumar et al. 2024). In the present work, we tried to elucidate the importance of probiotic microorganisms in strengthening brain plasticity changes during cognitive learning and memory formation with the help of a cue-based learning paradigm (CBLP).

2 Materials and Methods

2.1 Experimental Fishes

Commercially available adult goldfish (*Carassius auratus*) with body lengths (6-8 cm) and weights (8 – 15 g) were obtained from a local market. Obtained fishes were maintained with their respective groups (n = 6/group) in the home aquarium throughout the experimental period. Rectangular glass tanks, 42 X 30 X 21 (length, breadth, and height - LBH) inches, served as home aquariums. In the home aquarium, continuous air circulation, photoperiod (12 hours of light:12 hours of dark), and a standard temperature of 26 ± 2 ºC were provided for all experimental fishes. Commercial dry food pellets (Taiyo Pet Products India Pvt. Ltd) were provided thrice a day @ 8.00, 14.00, and 19.00 hours to meet the energy needs of all experimental animals. The home tank was adequately cleaned and replaced with fresh water on alternative days to maintain a debris-free environment. Experimental design follows the institutional ethical regulatory guidelines of Sri Ramakrishna Institutions, Coimbatore, Tamil Nadu, India.

2.2 Experimental Design

A rectangular glass tank (RGT) having an LBH of 42 X 30 X 21 inches was custom-designed and used in the behavioral analysis. The designed RGT was divided into three different compartments based on the study's needs. These three chambers include two feeding compartments (FC) and one central compartment (CC). FCs and CC vary in their LBH of 6 X 30 X 21 and 30 X 30 X 21 inches. In the two FCs, one FC acts as a positive reward chamber with blue colored cues, and another one acts as a negative chamber with red colored cues. Both the FCs have a central opening for fish movement from CC to FCs.

2.3 Infusion Mixture Preparation

All three probiotic strains *L. acidophilus* (MTCC No. 10307), *L. fermentum* (MTCC No. 9748), and *L. plantarum* (MTCC No. 12921) were acquired from the MTCC (Microbial Type Culture Collection and Gene Bank), IMTECH (Institute of Microbial Technology), Sector 39, Chandigarh, Punjab, India. Acquired probiotic strains were streaked on *Lactobacillus* MRS Agar to confirm their purity. After purity confirmation, acquired probiotic cultures were used to prepare an oral probiotic mixture in the ratio of 50:50 (as a single dose in a pure form), and 20:20:20:40 (as a single dose in mixed form). Prepared oral mixtures were used for the oral administrations into specific experimental groups after the primary phase of behavioral analysis (PPBA).

2.4 Behavioral Analysis

2.4.1 Experimental Groups

Fishes were randomly separated into four different groups. These are Group -1 (infused with *L. acidophilus*), Group – 2 (infused with *L. fermentum*), Group – 3 (infused with *L. plantarum*), and Group – 4 (infused with *L. acidophilus*, *L. fermentum*, and *L. Plantarum*).

2.4.2 Cue-based Learning Paradigm

The cue-based learning paradigm (CBLP) was used to understand the effect of a controlled environment and probiotic infusions on learning and memory formation (LMF). During LMF, two different color cues (blue, and red) were used for the behavioral studies in the FCs of the experimental apparatus. Blue and red colored cues act as positive and negative rewards with/ without food rewards. Behavioral responses were calculated based on the amount of time spent in the left chamber (LC), central chamber (CC), and right chamber (RC).

2.4.3 Predator Exposure Test

A predator exposure test (PET) was performed to identify whether probiotic oral infusions develop stress or not. In this PET, RGT has a size of 42 X 30 X 21 inches (LBH) and was used as an exposure chamber (EC). The EC was divided into three equal-sized zones (LBH of 14 X 10 X 21 inches), including a no-fear zone (NFZ), mid-fear zone (MFZ), and complete-fear zone (CFZ) with the help of two transparent plexi sheets with central openings. During PET, goldfish (*C. auratus*) and its predator (bluegills) were introduced into NFZ, & isolated chamber in CFZ for 15 minutes. Behavioral responses of all experimental groups were recorded in terms of time spent in NFZ, MFZ, and CFZ.

2.4.4 Open Field Test

Followed by PET, an open field test (OFT) is employed in this study to identify the presence/absence of anxiety-like behavior. OFT was carried out in RGT, which was 42 X 30 X 21 inches (LBH) and had 36 square boxes (5 X 5 cm/each). All 36 square boxes were divided into two different zones, i.e. the outer and inner zones. The outer and inner zone consists of 18 square boxes. Behavioural responses of experimental groups were analyzed in terms of time spent in an inner compartment (TSI) and time spent in an outer compartment (TSO).

2.5 Statistical Representation

Behavioral responses of all three behavioral tests were plotted as a bar diagram with the help of a Microsoft Excel program. An online tool (MedCalc statistical software) was used to calculate the p-value with the help of mean average values and standard error.

3 Results

3.1 Role of stress-free assimilated environment in the formation of cognitive functions

The current study initially uses the primary phase of behavioral analysis (PPBA) to understand the effect of a stress-free assimilated environment on cognitive memory formation using a CBLP in a controlled environment. Initially, animals of all experimental groups underwent learning and memory retention tests in the experimental apparatus after completing the assimilation and exploration phases. During the assimilation process, animals were maintained in the home tank for five days (Days 1-5) for their adaptations to the laboratory-controlled conditions. Following the habituation process, all experimental animals were allowed to explore the experimental apparatus without the color cue in FCs during the exploration phase (Days 6-8). Behavioral responses showed that initially, animals spent more time in CC than LC and RC. Later, time spent in CC was reduced in the subsequent days, showing that animals explored the FCs on the opposite sides of CC. After the exploration, all experimental groups were trained on CBLP based on color cues with/without food rewards between days 9-11. During the training phase, all experimental animals learned about the positive/negative stimuli based on food reward learning. Every visit to RC was awarded a food pellet, and vice versa, in LC. Obtained behavioral scores showed that the initial day (day 9) had fewer visits to RC than other days (days 10 and 11). It showed that animals spend more time in LC and CC on the first training day than in RC. Only a few animals grasped the information on the first day and were rewarded for their effort. Later, all animals gradually increased their visits to RC in the subsequent days due to training (Figure 1).

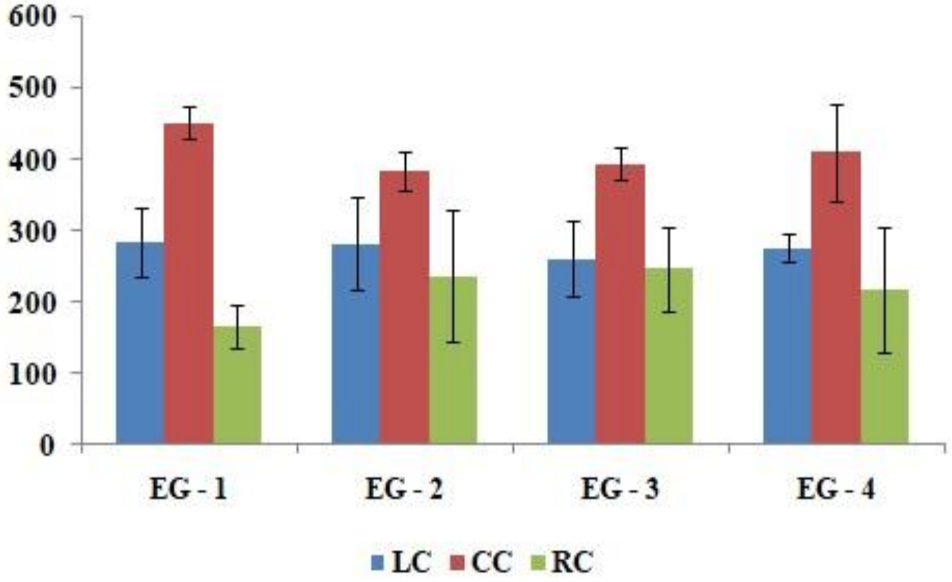

Figure 1 The first phase of behavioral training (without probiotic infusions - WiPI) showed that all experimental animals were trained in the experimental setup with the help of color cues based on reward learning. Initially, the number of visits to the right chamber (RC) was low on day 9 and gradually increased on days 10, and 11 which showed the animal's learning ability was associated with a reward. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

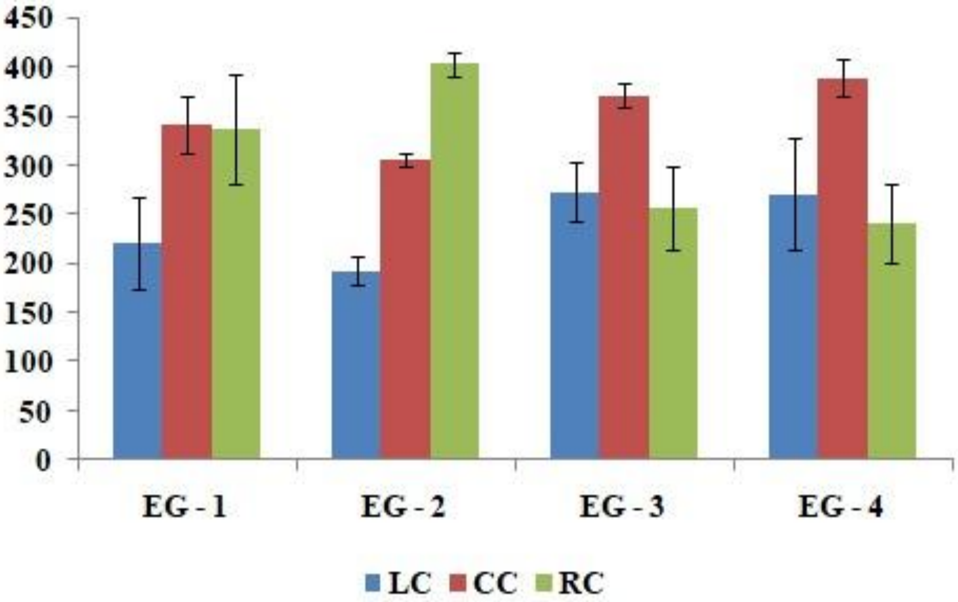

Figure 2 The first phase of behavioral testing (without probiotic infusions - WiPI) proved that experimental groups acquired information about the positive/negative reward with efficient retrieval of learned information. (Y axis denotes the time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

Following training, a five-day time gap (days 12-16) was given to consolidate learned information in the brain. Formed consolidated memory was tested for all experimental groups between days 17-20 in the experimental apparatus using positive and negative color cues. Behavioral scores proved that the training phase is important in retrieving learned information with an increased response to the positive chamber in the CBLP (Figure 2). Consolidated behavioral scores showed that a habituated stress-free controlled environment may involved in the formation of cognitive memory through the enrichment of formed neuronal plasticity (Figure 3). After completion of PPBA, all experimental groups are maintained in the home aquarium between days 21-23 for memory reconsolidation.

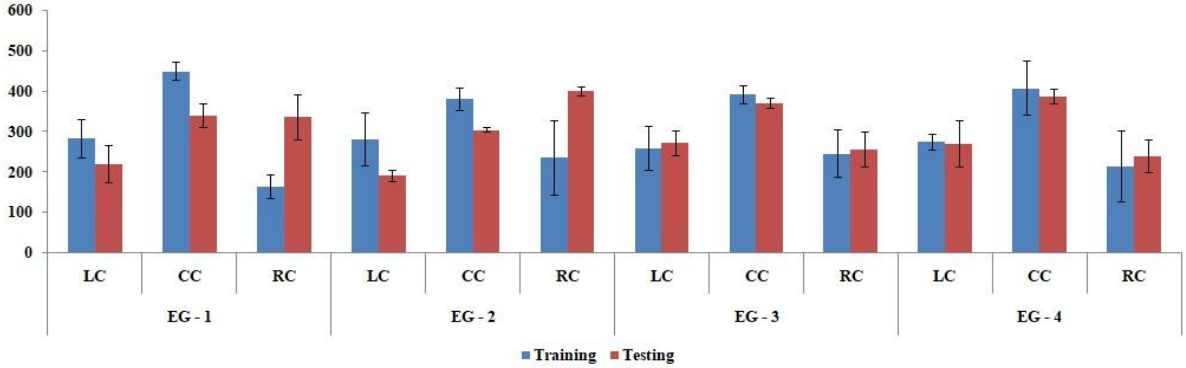

Figure 3 Comparative analysis of the primary phase of behavioral training and testing (without probiotic infusions - WiPI) showed that all four experimental groups learned about the provided positive/negative color stimuli during the process of training and retrieved stored information in an increased manner during the testing phase (Days 21 – 23). (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

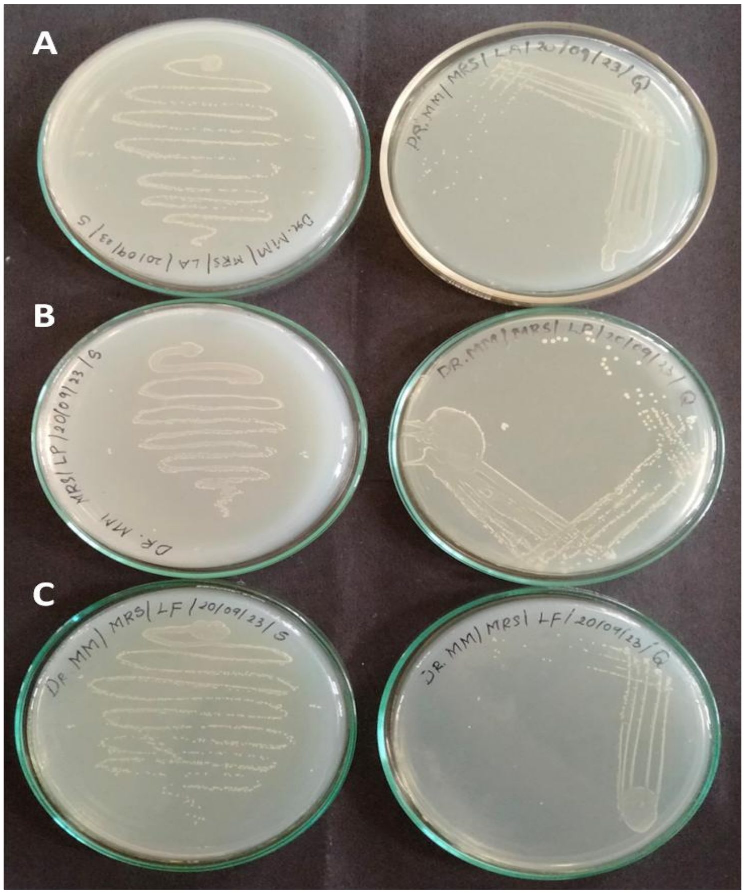

Figure 4 Representative plate pictures (A – *Lactobacillus acidophilus*, B - *Lactobacillus Plantarum*, and C - *Lactobacillus fermentum*) showing the purity confirmation of acquired *Lactobacillus* cultures with the help of the quadrant streak plate method.

3.2 Efficiency of probiotic infusions in the strengthening of developed cognitive functions

Following PPBA, the purity of acquired *Lactobacillus* cultures was identified using the quadrant streak plate method (Figure 4). Obtained individual colonies of all three probiotic microorganisms were inoculated in 5 ml of *Lactobacillus* MRS broth medium and incubated at 37 ºC for 24-36 hours. The prepared overnight culture was used for the preparation of the oral microbial mixture along with phosphate buffer saline (PBS) in a ratio of 50:50 (pure form)

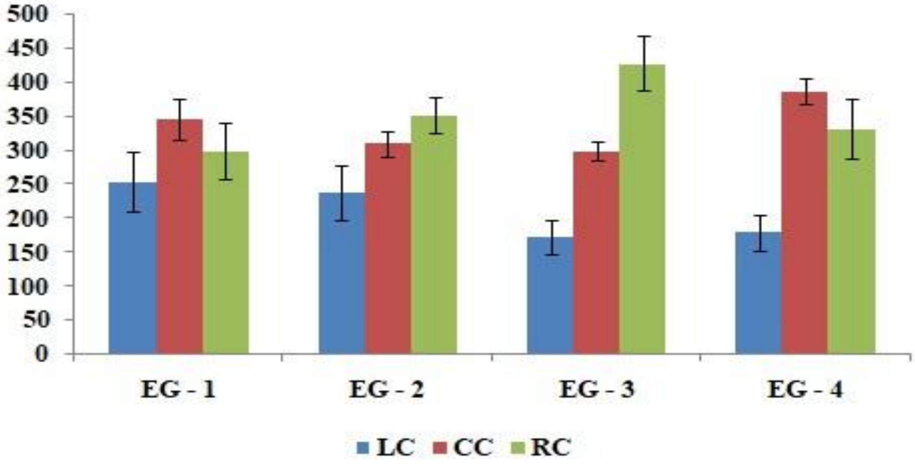

Figure 5 Behavioral scores of second phase training showed that probiotic oral infusions may take part in the strengthening of formed synaptic plasticity during the first phase of behavioral training. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

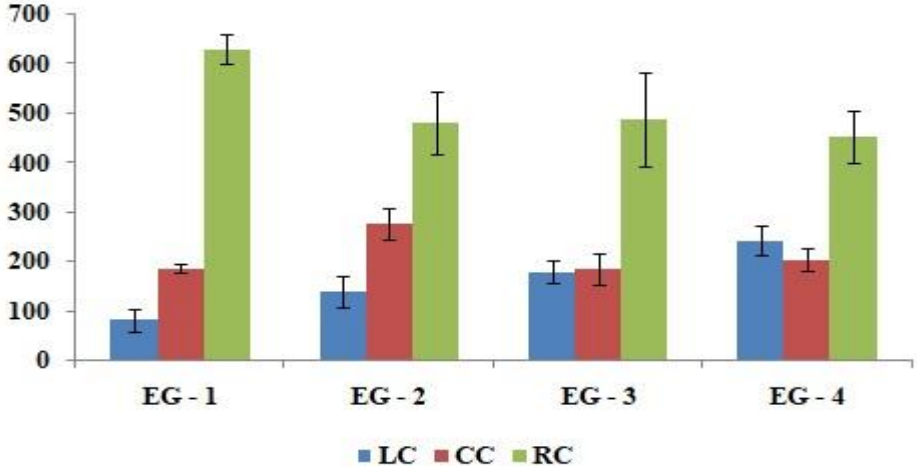

Figure 6 Behavioral scores of the second phase of testing showed that probiotic oral infusions also enhanced information retrieval compared to the first phase of behavioral testing. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

and 20:20:20:40 (mixed form). According to the study, the prepared infused mixtures were infused into experimental groups in pure and mixed doses. Before the secondary phase of behavioral analysis (SPBA), experimental groups – 1, 2, and 3 (received pure oral infusions) and experimental group – 4 are infused with mixed probiotic cultures. Prepared oral infusion mixtures were orally administrated into the experimental groups on day 24. Following the three-day time intervals (days 25-27), the SPBA training phase was carried out to identify the impact of probiotic oral microbial infusions (POMI) on strengthening synaptic connections during learning and memory formation. The training and testing phases were carried out between two different time intervals. In SPBA, training was carried out for three days between days 28-30. Behavioral responses of the SPBA training phase showed that all experimental groups actively learned about the reward provided in the RC compared to the FPBA training phase (Figure 5). SPBA training scores also showed that POMI may induce increased secretion of NPCs and neurotransmitters responsible for increased information acquisition. Followed by training, testing was carried out after 72 hours (3 days) of SPBA training on days 34-36. Testing behavioral scores showed that all animals spent more time/a higher number of visits to the positive-reward chamber than other chambers (Figure 6). Comparative analysis of training and testing phases proved that POMI played a major role in strengthening formed plasticity changes in the brain (Figure 7).

Comparative analysis of two different training phases, PPBA (without probiotic infusions – WiPI) and SPBA (with probiotic infusions – WPI), proved that a controlled habituated laboratory environment plays an unavoidable role in the formation of stress-free cognitive memory

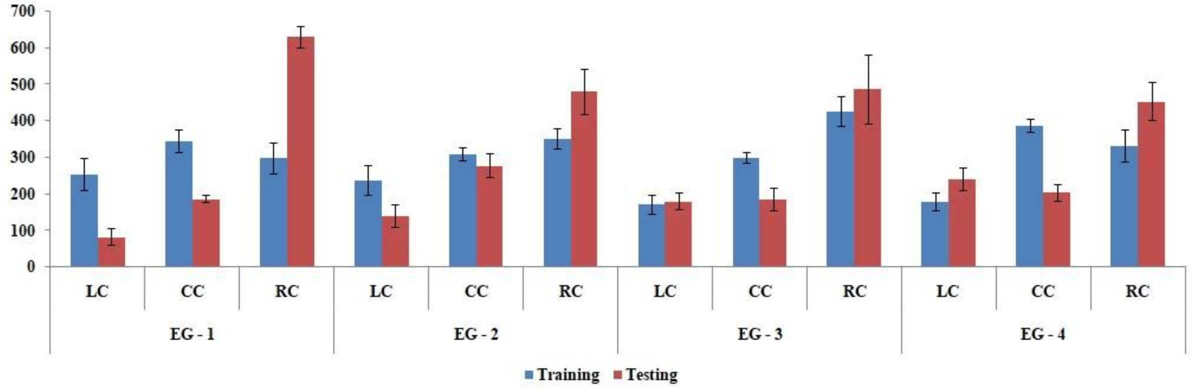

Figure 7 Comparative analysis of behavioral training and testing (with probiotic infusions) showed that all four experimental groups showed enhanced learning abilities during the process of training and efficient retrieval of stored information compared to the without probiotic infusive training and testing. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

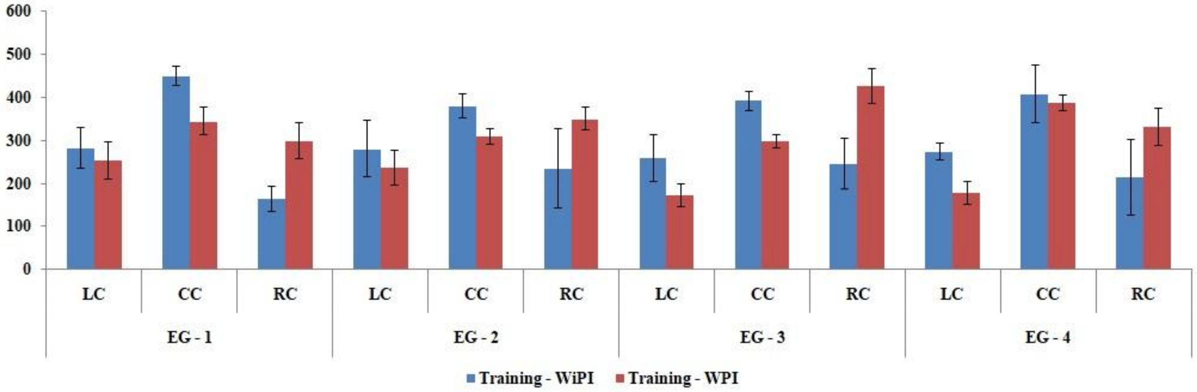

Figure 8 Comparative analysis of behavioral training (without probiotic infusions – WiPI Vs with probiotic infusions – WPI) showed that probiotic oral infusions played a major role in the development of synaptic plasticity (enhanced learning ability) compared to non-infusive training. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

through the formation of neuronal/synaptic plasticity changes. Formed neuronal plasticity was further strengthened during the training phase after probiotic infusions. Thus, the obtained results showed that formed plasticity changes can be strengthened with the help of probiotic intake (Figure 8). Besides enhancing learning ability, probiotic oral infusions also enhance the efficient retrieval of learned information during testing after infusions. Thus, the comparative testing analysis proved that probiotic microorganisms play a pivotal role in strengthening formed neuronal connections with their intake in pure and mixed form (Figure 9).

3.3 Effect of probiotic oral microbial infusions on the development of stress-free cognitive functions

To prove the effect of probiotic oral microbial infusions on stress-free memory development, we used predator exposure test (PET) and open field test (OFT) in this study. All experimental animals performed PET and OFT to detect the absence of anxiety-like behavior, and fear memory formation in the infused groups. Scores of PET showed that probiotic oral microbial infusions were never involved in the formation of anxiety-like behavior in infused groups. The animals spent more time in the stress-free zone (inner compartments) than the stressed zone (outer compartments) in the experimental setup. It also proved that probiotic oral microbial infusions never induce anxiety-like behavior against pure and mixed doses of infusions (Figure 10). Followed by PET, OFT was performed to prove that probiotic oral infusions may prevent the formation of fear memory when experimental groups are exposed to their predators. Behavioral responses of the experimental groups showed that all animals spent more time near their predator, which showed that probiotic oral infusions might limit the production of cortisol through the hypothalamic-pituitary-adrenal (HPA) axis. Reduced amount of cortisol production may result in the prevention of stress formation (Figure 11). Statistical analysis showed no significant differences among the CBLP, PET, and OFT experimental groups. Nonsignificant differences were calculated by the observed p values with the help of mean average values and standard errors.

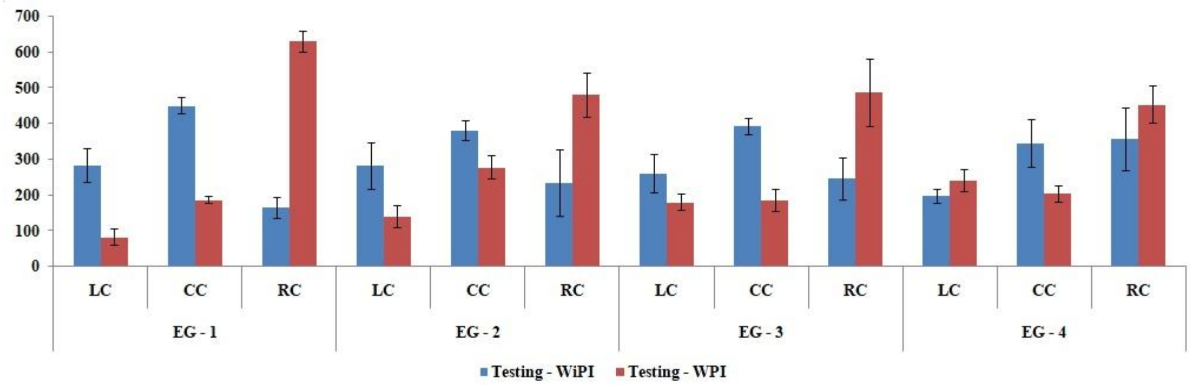

Figure 9 Comparative analysis of behavioral testing (without probiotic infusions – WiPI Vs with probiotic infusions – WPI) showed that probiotic oral infusions played an unavoidable role in the retrieval of learned information along with the development of synaptic plasticity during the testing phase. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

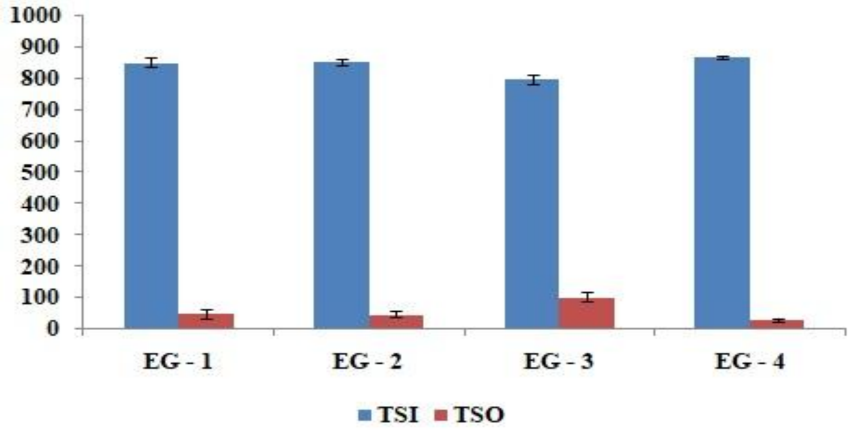

Figure 10 Behavioral responses of open field test (OFT) showed that infused experimental groups with probiotics in pure (EG – 1, 2, and 3) and mixed form (EG – 4) did not show any anxiety-like behavior development as animals spent more amount of time in the inner compartment (TSI) compared to the outer compartment (TSO). (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

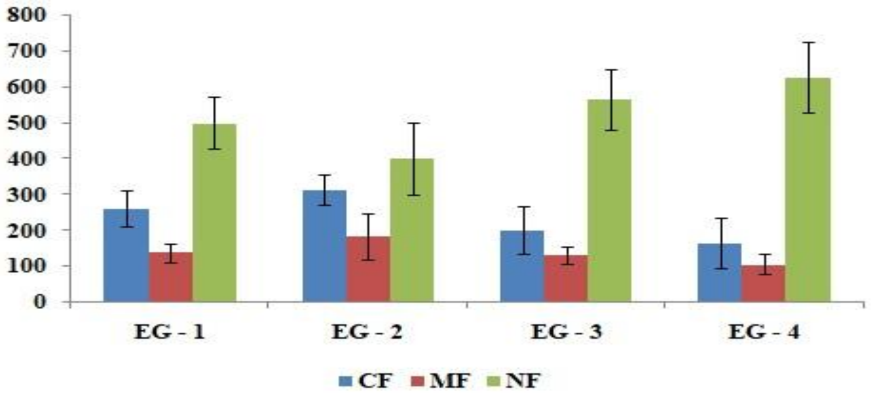

Figure 11 Behavioral scores of the predator exposure test (PET) proved that probiotic oral infusions do not show any fear memory development in the experimental groups. Responses showed that all experimental animals spend most of the time near the predator [No fear zone (NF)] other than mid fear zone (MF), and complete fear zone (CFZ). Thus observed result supports the role of probiotics in the alleviation of stress formation. (Y axis denotes time in seconds; EG – experimental group; LC- left chamber; RC – right chamber; CC – central chamber).

4 Discussion

In recent scenarios, oral and gut microflora research has become a prominent research area in the oral-gut-brain (OGB) axis. This OGB axis shows close interconnection between the oral cavity, brain, and gastrointestinal tract (GI) (Narengaowa et al. 2021; Paudel et al. 2022; Chaudhry et al. 2023; Mukilan et al. 2024b). Emerging clinical evidence shows that this oral and gut microbiota may contain many bacterial communities, including beneficial and harmful flora. In a healthy condition, these beneficial flora play a relevant role in the maintenance of the homeostasis mechanism in the GI, which results in the production and transmission of neurotransmitter precursor compounds (NPCs) from the gut to the CNS (Zheng et al. 2020; Chen et al. 2021; Varela-Trinidad et al. 2022; Ji et al. 2023). The BBB carried out transmission of NPCs through the vagus nerve in a direct/indirect way. Transmitted NPCs produce brain neurotransmitters and the subsequent activation of neuronal signaling molecules involved in cognitive memory formation (Alajangi et al. 2022; Sarubbo et al. 2022; Mukilan 2023). Dysbiosis of oral/gut microflora further plays a major role in forming immune dysfunction, lung diseases, and reduced cognitive functions within a host. Few reports showed that ageing, food habit alterations, pathogenic infections, and poor oral hygiene play an unavoidable role in the development of cognitive dysfunctions through oral-gut dysbiosis (Kandpal et al. 2022; Malik et al. 2023; Mitra et al. 2023). Formed oral-gut dysbiosis consequently results in the causation of gastritis and other systematic disorders. This formed dysbiosis state can be reversed by the intake of probiotic microorganisms along with diet for the maintenance of probiotic flora within the oral cavity and gut (Sandhu et al. 2017; Den et al. 2020; Zhu et al. 2021; Liu et al. 2023).

The present study strived to explore the role of probiotic microorganisms in developing and strengthening synaptic plasticity with the help of a cue-based learning paradigm. Recent reports have proved that pathogenic microbial colonization plays a significant role in developing cognitive dysfunctions during dysbiosis. In healthy conditions, this pathogenic microbial colonization may controlled by the action of native flora with its mutual relationship with the barrier immunity of the existing host immune system (Sarkar et al. 2021; Dash et al. 2022; Ahmed et al. 2024; Pitchaikani et al. 2024). To prevent the colonization of pathogens, probiotic intake has become an alternative way to safeguard the host from oral and gut microflora disturbances. Based on the information availed from the recent reports, the current study tried to traverse through the beneficial effect of probiotics in the strengthening of brain neuronal plasticity changes (Han et al. 2021; Wang et al. 2021; Dahiya and Nigam 2022; Gebrayel et al. 2022). Obtained results showed that probiotic oral infusions had a major role in regulating neurotransmitter production and its associated signaling molecules for the development of proper cognitive memory. Formed cognitive memory showed the proper production and transmission of NPCs from the gut to the brain via the BBB. Transported NPCs resulted in synthesizing neurotransmitters (serotonin) in the presynaptic neurons and their release into the SC. Once released into the SC, serotonin binds with 5-HT receptors, increasing calcium influx inside postsynaptic neurons. Increased calcium influx further activates neuronal signaling molecules needed for the maintenance of proper cognitive health (Chen et al. 2021; Dicks 2022; Miri et al. 2023; Margoob et al. 2024). This probiotic strain may also prevent stress formation by regulating the production of cortisol in the hypothalamic-pituitary-adrenal (HPA) axis (Freimer et al. 2022; Sabit et al. 2023). Thus, the present study revealed that probiotics are responsible for cognitive memory formation in the brain and alleviating stress formation in a host system.

Conclusion

The eventual upshots of the present study manifest the effect of probiotic infusions on the formation of cognitive memory. Recently, it was shown that pathogenic microbial colonization or conversion of normal flora into opportunistic pathogens may result in cognitive impairment through oral/gut dysbiosis. It was also reported that formed oral/gut dysbiosis can be reversed with the oral intake of probiotic microorganisms to reverse the formed cognitive impairment. In the current study, the ramifications of probiotics on the formation and strengthening of neuronal connections were studied in a controlled, habituated, serene environment. The studies showed that probiotic’s impact on enhancing brain plasticity development was studied using a reward-based learning paradigm in a laboratory. Experimental behavioral responses showed that entering live probiotic microorganisms into the oral passage might prevent pathogenic colonization in the oral cavity and gut and positively impact cognitive health. Development of cognitive health results in the proper neurotransmitter production by transmitting the needed amount of neurotransmitter precursor compounds from the gut through the BBB. Formation of the needed quantity of neurotransmission results in the development of RNA/Protein mediated LT memory formation by regulating neuronal signaling molecules, microRNAs, and negative regulators of cognitive memory formation. It also proved that probiotics may control the synthesis and production of stress hormones like cortisol in the HPA axis other than enhanced neuronal plasticity changes. For the first time, the current study opened up the dual role of probiotic microorganisms in controlling stress formation and its effect on developing and strengthening existing neuronal connections to maintain proper cognitive health. The present study also revealed that probiotics can be stress relievers to control endocrine hormone production.

Authors Contributions

MM performed the conceptualization, research design, funding acquisition, original investigation, draft preparation, revision, and editing of this manuscript. RA and SP performed the experimentation and data collection.

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Conflict of Interest

Authors report no conflicts of interest in this work

Data Availability

Research data is available with the authors and shall be provided upon request.

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