Impact of Antibiotic Oral Infusions on the Development of Brain Cognitive Dysfunctions

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Abstract

Cognitive impairment was observed as a serious mental health problem ranging from mild cognitive loss to dementia. This cognitive impairment was stated as impairment in the gut microbiota-brain communication. In a healthy state, gut microbiota plays an unavoidable role in the regulation of cognitive functions like memory formation, anxiety, stress and mood. However, the stated cognitive functions may be hindered by the aberration of gut microbiota through pathogenic colonization/continuous antibiotic intake during disease conditions. Imbalance in this gut microflora further results in the development of oral/gut dysbiosis. Formed oral/gut dysbiosed state shows a decrease in the production of neurotransmitter precursor compounds (NPCs) in the gut, which has an impact on impaired cognitive memory formation. Recent reports have shown that colonization of periodontal/non-periodontal pathogens results in the formation of cognitive decline during dysbiosed state in oral/gut microbiota. The present study made an initial attempt to study the impact of antibiotic oral intake on cognitive memory formation through oral/gut dysbiosis. A comparative two-phased behavioral analysis [primary phase of behavioral studies (PPBS) and secondary phase of behavioral studies (SPBS)] was used to study the impact of antibiotics on the development of cognitive dysfunctions based on a reward-based learning paradigm (RBLP).

Results of the present study showed that gentamicin sulphate oral infusions result in the development of cognitive impairment through the formation of oral/gut dysbiosis. Obtained behavioral scores showed that repeated antibiotic intake may result in the development of cognitive dysfunctions through oral/gut dysbiosis. The formed oral/gut dysbiosis results in the development of impaired LTM formation in the infused behavioral study groups compared to non-infused behavioral study groups. Thus the present study proved that intake of antibiotics in a higher concentration or for a longer period may result in the development of cognitive dysfunctions.

Keywords: Cognition, Gentamicin, Long-term memory formation (LTM), Reward-based learning paradigm (RBLP).

Introduction

Oral and gut microbiotas refer to the trillions of microorganisms present in the gastrointestinal (GI) tract of a host. These microbiotas consist of bacteria, viruses, protozoa and fungi and act as virtual organs of a host system. The microbiota composition is determined by environmental factors like diets and drugs to a larger extent[16,44,53]. Nowadays, extended use of antibiotics is more common among young, middle-aged persons for the treatment of illnesses like sore throat, cough and fever. Repeated use of a certain kind of antibiotic may result in the development of antimicrobial resistance (AMR) within the host[21,49]. The development of AMR results in the increase of pathogenic population within the gastrointestinal tract. An increase in pathogenic load further reduces the amount of beneficial flora present in the oral cavity and gut. This reduced microbiota results in the formation of oral/gut dysbiosis[6,9,18,19].

In healthy conditions, native microfloras of the GI tract were responsible for the formation of bidirectional communication between the gut and central nervous system (CNS). The formed bidirectional communication was facilitated by the enteric nervous system through the vagus nerve. This communication was initiated by the synthesis of neurotransmitter precursors (NPs) in the form of primary/secondary metabolites from the gut[17,47,54]. The formed primary/secondary metabolites were transported from the gut through the blood-brain barrier by the induction of the vagus nerve. The gut-formed NPs act as precursor molecules in the synthesis of certain kinds of neurotransmitters within the brain[4,7,46].

Recent studies have shown the importance of gut microbiota in the synthesis of NPs needed for the synthesis of brain neurotransmitters like serotonin (5-HT), γ-aminobutryic acid (GABA) and dopamine (DA)[10,13]. Among the three stated neurotransmitters, 5-HT plays a major role in the development of cognitive functions in the brain. This 5-HT neurotransmitter was produced from an amino acid precursor molecule called tryptophan[5,10,25]. This tryptophan can be entered into the host system in the form of a dietary supplement. The dietary intake of banana, bread, oats, fish, peanuts, chocolate, milk and its derivatives like cheese act as potential sources for the synthesis of 5-HT in the brain through the supply of tryptophan to the gut microbiota[23,48,56].

Entered tryptophan is further converted into derivatives of indole compounds within the gut. These conversions were taking place with the help of *Escherichia coli* and *Clostridium perfringens* into L-tryptophan and other

derivatives like indoxyl sulfhate, 6-sulfate, indole acetylglycerine and indole-3-propionate[1,34,55]. Later on, produced 5-HT is converted into 5 – hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TH). After conversion, 5-HTP crosses the BBB through the enteric nervous system (ENS) and reaches the brain[7,13,25].

Once reaching the brain, 5-HTP is converted to 5-hydroxytrptamine (5-HT) with the help of an enzyme aromatic l-amino acid decarboxylase. Produced 5-HT is released into the synaptic cleft for its binding with the specific 5-HT receptors in the synaptic cleft (SC)[10,13,15]. Unbound 5-HTs present in the SC were further taken by the pre-synaptic neuron for its degradation by the monoamine oxidase (MAO) into 5-hydroxytryptophol and 5-hydroxyindole-3-acetic acid with the help of alcohol-dehydrogenase (ADH) enzyme. Specific binding of 5-HT with the post-synaptic neuronal receptors results in the activation of the CREB-mediated neuronal signaling pathway as a result of increased calcium influx within the post-synaptic neuron (PSN).

Activation of the CREB-mediated neuronal signaling pathway results in the formation of cognitive memory in a habituated environment with the help of various neuronal signaling molecules[12,29,31,33,40,51,52]. Based on the available research findings, the present study attempted to show the effect of antibiotic intake in the formation of cognitive memory impairment through the induction of oral/gut dysbiosis. Reward-based learning paradigm (RBLP) was used to study the brain activity of experimental groups (EGs) with the help of two different color cues which may act as positive and negative stimuli for learning and memory formation based on a food reward.

Material and Methods

Study Animals: Commercially available goldfish (*Carassius auratus*) used in this study were purchased from a local aquarium in Coimbatore, Tamil Nadu, India. Purchased fishes were measured for their body length (BL) (4.5 – 6 cm) and weight (W) (6 – 8 g) once they reached laboratory conditions without any transport stress. After fulfilling the study requirements (BL and W), study animals were assigned to the respective behavioral study groups (BSGs). All BSGs were maintained in a laboratory aquarium having an ambient temperature (26 ± 2º C), light and dark cycle (12:12 hours) and continuous aeration (24 X 7/day) for their adaptation to the laboratory conditions. The adaptation period is also called as habituation phase which is given between days 1- 5.

During the habituation phase (days 1- 5), animals were fed with commercially available dry round food pellets thrice a day at the time intervals of 09.00, 14.00 and 18.00 respectively. Aquarium water was replaced on alternative days to maintain a debris-free environment without microbial conditions. The experimental design and protocols used in this study followed the institutional ethical guidelines of Sri Ramakrishna Institutions, Coimbatore – 641 006, Tamil Nadu, India[27].

Study Design: The study design was planned according to the need of the behavioral study [reward-based learning paradigm (RBLP)]. In this study, a rectangular glass tank having a size of 42 (length) X 30 (breadth) X 21 (height) inches was used as an experimental chamber (EC). Designed EC consists of three different chambers including one central chamber (CC) and two feeding chambers (FCs). FC is located at the left and right side of the EC with CC at the centre. Designed CC and FCs (LC and RC) are having a size of 30 X 30 X 21 and 6 X 30 X 21 inches (length, breadth and height) respectively[28].

Behavioral Study Groups: Behavioral studies were carried out for all five BSGs (BSG - 1, BSG - 2, BSG - 3, BSG - 4 and BSG – 5) in the present study with the help of RBLP. Behavioral studies were carried out in two different phases. They are termed the primary phase of behavioral studies (PPBS) and the secondary phase of behavioral studies (SPBS). In PPBS, all five BSGs were allowed to perform all behavioral parameters (exploration, training and testing) without any oral infusions. Compared to PPBS, four BSGs (BSG - 2, BSG - 3, BSG - 4 and BSG - 5) received antibiotic oral infusions (AOI) compared to BSG – 1 which acted as a control (non-infused group) in SPBS.

Oral Infusion Mixture Preparation: Antibiotic gentamicin sulphate (CMS461-1G) was purchased from Himedia Laboratories Pvt. Ltd., India. Purchased antibiotic powder was used for the preparation of oral infusion mixtures in the concentrations of 20, 40, 60 and 80 microgram/microlitres with the help of phosphate buffer saline (PBS) solution. The antibiotic oral infusion (AOI) mixtures were prepared freshly on the infusion day.

Behavioral Analysis: To test the impact of gentamicin sulphate oral infusions on BSGs, all BSGs underwent a comparative two-phased behavioral analysis with the help of PPBS and SPBS. During PPBS, all five BSGs undergo behavioral parameters like exploration, training and testing without any AOI. Followed by PPBS, SPBS was performed after infusing BSGs with different concentrations of antibiotics as oral mixtures.

Primary Phase of Behavioral Studies: Initially, PPBS was performed in the EC with the help of RBLP. For this RBLP, two different color cues (blue and red) were used as positive and negative stimuli. Blue color cues act as positive stimuli and red color cues are designated as negative stimuli. Positive and negative stimuli were presented to the study animal in the FCs. Positive stimuli were associated with a food reward and vice versa in negative stimuli. In PPBS, exploration (days 6-8), training (days 9-11) and testing (days 15-17) phases were performed by the study animal to find their object recognition ability, acquaintance of new information and retrieval of learned information. Three –

day time interval between the training and testing phases was given for the memory consolidation within the brain regions.

Secondary Phase of Behavioral Studies: After completion of PPBS, four SGs (BSG - 2, BSG - 3, BSG - 4 and BSG - 5) received AOI in the concentrations of 20, 40, 60 and 80 microgram/microlitres with the help of an oral gauge. Followed by infusions, BSGs were provided with 72 hours of resting time for the settlement of AOI in the gut (days 18-20). Followed by resting time, all BSGs (both infusive and non-infusive) were trained in the EC for three-days (days 21-23) with the same setup used in the PPBS. Followed by training, 72 hours of time gap (days 24-26) was given to SGs for the memory consolidation process. Finally, the testing phase was carried out between days 27-29 to test the impact of AOI on cognitive functions with the help of RBLP.

Predator Exposure Test: The predator exposure test (PET) was performed to identify the development of fear memory after AOI. In PET, glass EC with a size of 42 X 30 X 21 inches was used for this study. The EC was divided into three chambers with a length, breadth and height of 14 X 10 X 21 inches. These three chambers include the complete fear zone (CFZ), mid-fear zone (MFZ) and fearless zone (FZ). *Bluegills* were used as a predator and placed in the isolated chamber present within the FZ.

Open Field Test: An open field test (OFT) was performed to study the effect of AOI on the development of anxiety-like behavior. A glass rectangular tank having a length, breadth and height of 42 X 30 X 21 inches was used for the study. The bottom of the tank was divided into 36 individual boxes with a size of 10 X 5 cm/each. OFT was performed after the completion of the SPBS training and testing phases.

Statistical Representation: Behavioral scores of all behavioral paradigms (PPBS and SPBS) were used for the preparation of the bar diagram using Microsoft Excel Program.

Results

The present study uses two different behavioral paradigms (PPBS and SPBS) to identify the impact of gentamicin sulphate oral infusions on the induction of oral/gut dysbiosis and its effect on impaired cognitive memory formation. All BSGs performed PPBS without any oral infusions in a habituated stress-free environment to identify the impact of residential oral/gut microbiota on cognitive memory formation. Following PPBS, the training and testing phases of SPBS were performed by the non-infusive group (BSG – 1) and infusive groups (BSG - 2, BSG – 3, BSG – 4 and BSG – 5. Infusive groups received antibiotic (gentamicin sulphate) oral infusions after completion of the PPBS testing phase.

Role of Primary Phase of Behavioral Studies in the Identification of Cognitive Memory Formation in a Stress-Free Environment: The primary phase of behavioral studies (PPBS) starts after the completion of the habituation process between days 1-5 in the laboratory aquarium by all BSGs. Following the habituation process, an exploratory task was given to all BSGs between days 6-8 in the EC without color cues in the FCs. On day 6, all BSGs spent more amount of time in the CC without exploring the FCs located on the opposite sides of EC. Later on, BSGs spent more amount of time either in one of the FCs or in both of them with frequent visits by crossing the CC on days 7 and 8. Behavioral scores of the exploration phase showed that all BSGs initially had an oscillation to explore the different regions of the EC. It may be due to the new environment with three different chambers.

On consecutive days, all BSGs used navigation cues to explore all regions of the ECs actively (Fig. 1). The outcome of the exploratory phase showed that all the BSGs were active in their physiological state to grasp the new information with a food reward provided in the EC during the training phase.

Continuation of the exploration phase results in the behavioral training of all BSGs in the EC with the help of RBLP. The training phase was carried out between days 9 and 11 to understand the learning ability of all SGs in a comparative manner. On the day 9, animals of all BSGs were introduced into the EC for 900 seconds (15 minutes) as individual ones. Initially, BSGs spent more amount of time in CC and LC compared to RC. On days 10 and 11, it was vice-versa in all SGs. It may happen due to the animal's learning ability to find the positive cue with a food reward. Time spent in RC was gradually increased from the first day of training (day -9) as a result of learning with a reward attaining its maximum time spent/entry to RC in the final day of training (day – 11).

Behavioral responses of all BSGs were recorded and were used for the calculation of time spent in the CC, LC and RC. During the training phase, positive and negative color cues were placed in the RC and LC. Individual behavioral responses of all BSGs showed that all animals grasped the new positive stimuli provided in the EC with a food reward (Fig. 2). All BSGs were awarded dry round food pellets as a reward for their effort to identify the determined positive color cue.

Finally, testing was carried out in the PPBS after a three-day time interval provided to all BSGs between days 12 and 14. This interval time (72 hours) was given for the memory consolidation process to take place in the hippocampus region of the brain. Behavioral responses of the testing phase showed the maximum number of entries to the positive reward chamber which proves the strength of memory consolidation formed in all BSGs. During the testing phase, positive stimuli were placed at LC to identify the retrieval of learned information in the BSGs. Initially on day 12, more number of visits to RC was high compared to CC and LC. Later on, it was gradually decreased on RC which is having

negative stimuli without a food reward. On the later days (days 13 and 14), all BSGs had a more number of visits to the LC for getting a food reward. Each visit was rewarded with a food pellet during the testing phase.

Behavioral responses of BSGs showed that all animals retrieved the learnt information in an increased manner during the testing period which showed the impact of training in a stress-free environment (Fig. 3). Observed results also proved that learned information was stored in the hippocampus without any spatial cues. Thus the PPBS proved that training and testing phases were responsible for grasping new information, learned information storage and its retrieval in a stress-free habituated environment (Fig. 4). Further, it coincides with the role of oral/gut microbiota in the development of cognitive functions and maintenance of brain homeostasis mechanisms.

Effect of Antibiotic Oral Infusions on the Development of Impaired Cognitive Memory Formation: The secondary phase of behavioral studies (SPBS) was carried out after the completion of PPBS on day 21 with the training and testing phases to prove the effect of gentamicin sulphate oral infusions on cognitive memory formation. After PPBS, four BSGs (BSG - 2, BSG - 3, BSG - 4 and BS - 5) were infused with antibiotic concentrations of 20, 40, 60 and 80 micrograms/microlitres with the help of an oral gauge. Followed by infusions, a three-day resting time (days 18-20) was given for the infusive group along with the non-infusive group (BSG - 1). SPBS training was performed by all BSGs between days 21-23 in the same EC used for the PPBS. Behavioral responses of the infusive groups (BSG - 2, BSG - 3, BSG - 4 and BS - 5) showed that initially (on day 21) AOI did not have a greater impact on the information acquaintance during the training phase of SPBS (Fig. 5).

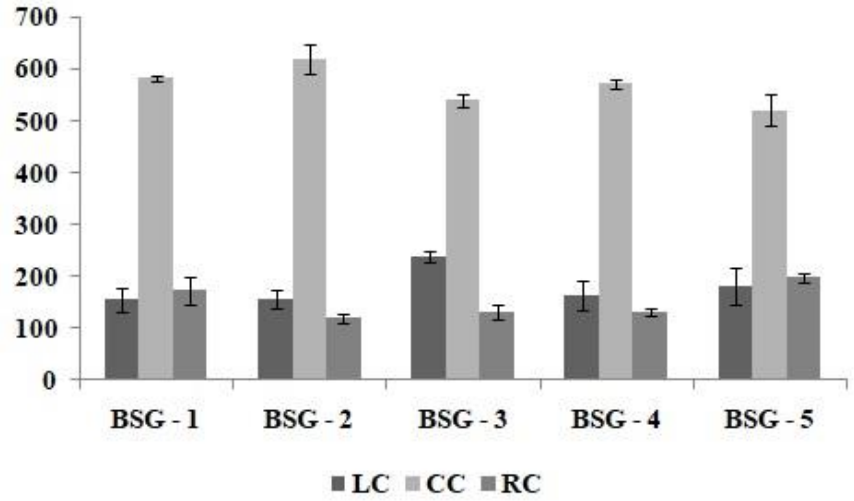

Figure 1: Behavioral responses of the primary phase of behavioral studies (PPBS) exploration phase showed that all experimental animals of behavioral study groups (BSGs) were active and spent more amount of time in the central chamber (CC) compared to the other two feeding chambers (left chamber – LC and right chamber – RC)

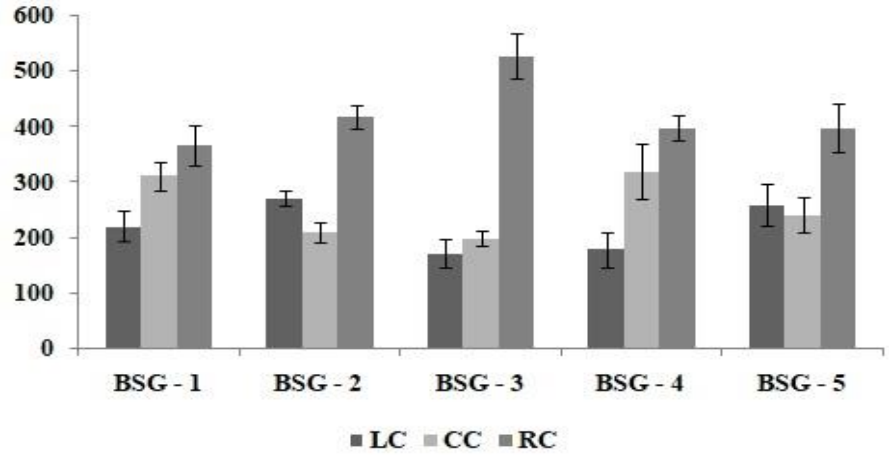

Figure 2: Behavioral scores of the primary phase of behavioral studies (PPBS) training phase showed that the number of attempts was increased in the right chamber (RC) compared to the central chamber (CC) and left chamber (LC). Obtained behavioral responses showed that the number of attempts to the positive reward chamber (right chamber) was increased due to the learning ability of behavioral study groups (BSGs) in the consecutive days compared to the negative reward chamber (LC)

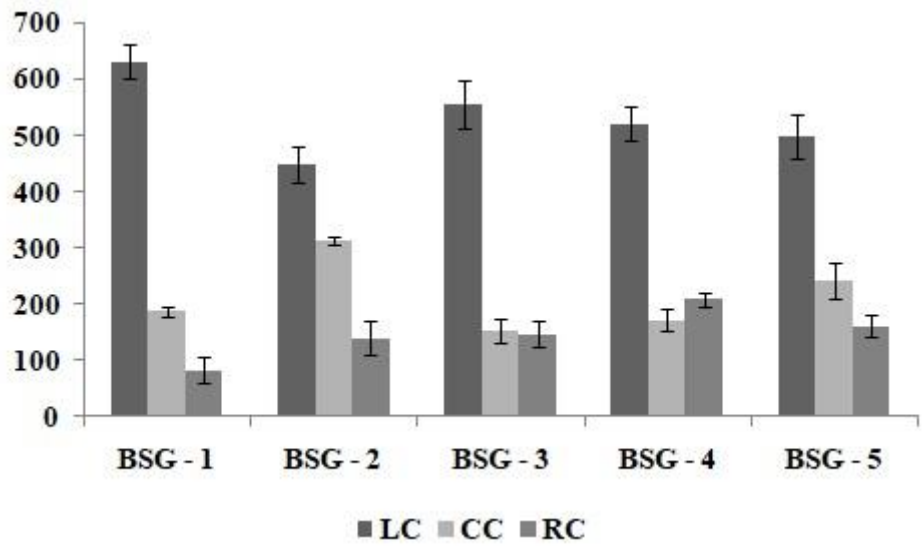

Figure 3: Behavioral scores of the primary phase of behavioral studies (PPBS) testing phase showed that the number of attempts was increased to the left chamber (LC) compared to the central chamber (CC) and the right chamber (RC). Obtained behavioral responses showed that the number of attempts to the positive color cues (LC) was increased due to the memory retrieval of behavioral study groups (BSGs) during the study period compared to the PPBS training phase

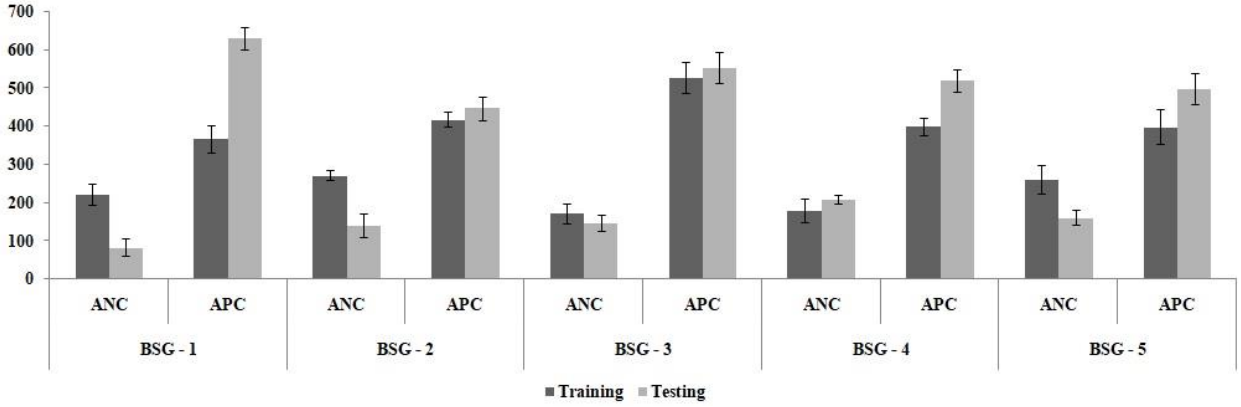

Figure 4: Represented bar diagram showing the behavioral responses of training and testing phases of the primary phase of behavioral studies (PPBS). Behavioral scores showed that the number of attempts to the positive chamber (APC) was increased during the testing phase compared to the training phase which shows stronger memory consolidation in the brain regions (BSG - Behavioral study group; ANC - Attempts to the negative chamber; APC - Attempts to the positive chamber). To avoid spatial learning, positive color cues were placed in alternative feeding chambers during the training and testing phases

Collective behavioral scores of SPBS training phase showed that gentamicin sulphate may have a lesser/null impact on the animals learning ability in the EC. Completion of the SPBS training phase results in the initiation of the testing phase between days 27 and 29. SPBS testing follows the experimental setup of the PPBS testing phase with the change in the positive reward chamber. Behavioral responses of SPBS testing showed very few numbers of visits to the RC acting as a positive reward chamber compared to the LC and CC.

Observed behavioral data also proved that antibiotic infusion causes major hindrances in the learning ability and its retrieval through the increase of AMR which finally results in the disruption of oral/gut dysbiosis (Fig. 6). Comparative analysis of SPBS training and testing phases showed that AOI may have a mild/null impact on the learning abilities of the infusive BSGs compared to non-infusive BSG. However, AOI showed a greater reduction in the memory retrieval of learned information which showed the development of impaired cognitive functions in the antibiotics-infused BSGs. Obtained results proved that oral antibiotic intake may be involved in the development of cognitive impairment through the reduction of oral and gut native flora via oral/gut dysbiosis (Fig. 7).

Comparative analysis of PPBS and SPBS training phases showed that antibiotic gentamicin sulphate had a minimum level of hindrances in the learning abilities of all BSGs compared to the non-infusive group (SG - 1). Obtained

results proved that oral/gut dysbiosis had a minimal effect on the acquisition of new information in the infusive BSGs (Fig. 8).

Compared to the training phases, PPBS and SPBS testing phase scores showed that AOI may have a greater impact on the retrieval of learned information in the infusive BSGs compared to the control (non-infusive BSGs) (Fig. 9). Collectively PPBS and SPBS training and testing scores showed that AOIs may have a greater impact on the memory consolidation process taking place in the brain as a result of oral/gut dysbiosis or neuronal inflammation in the brain regions.

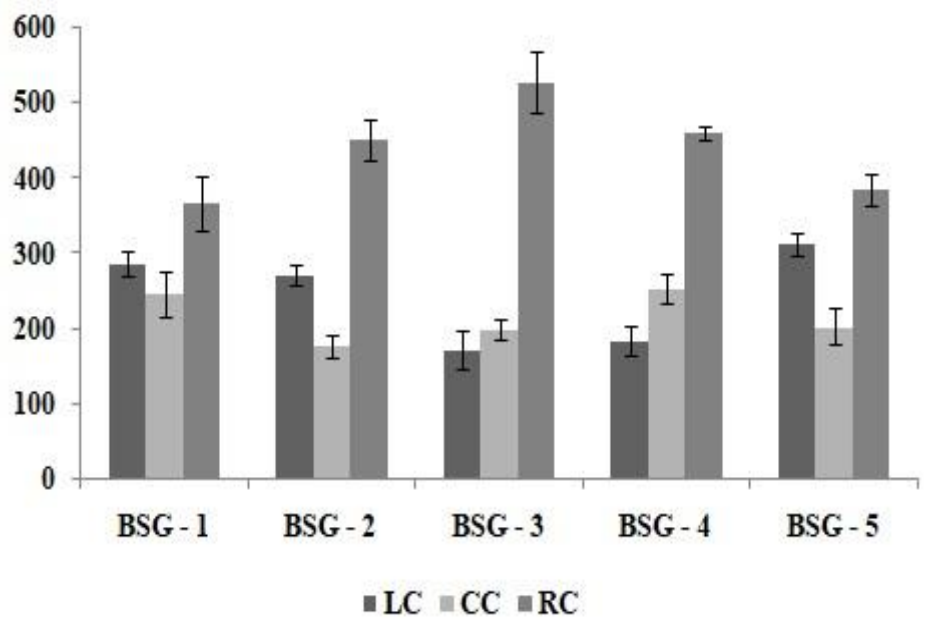

Figure 5: Behavioral responses of the secondary phase of behavioral studies (SPBS) training phase showed the antibiotic oral infusions (AOIs) did not have any impact on the animal's learning ability in the infusive groups compared to the control. The number of attempts was increased in the positive reward chamber (right chamber - RC) compared to the negative reward chamber (left chamber-LC). Obtained behavioral scores showed that the AOI was not involved in the destruction of cognitive functions in infusive groups compared to control (non-infusive group) (BSG - Behavioral study group; LC - left chamber; CC - central chamber; RC - right chamber

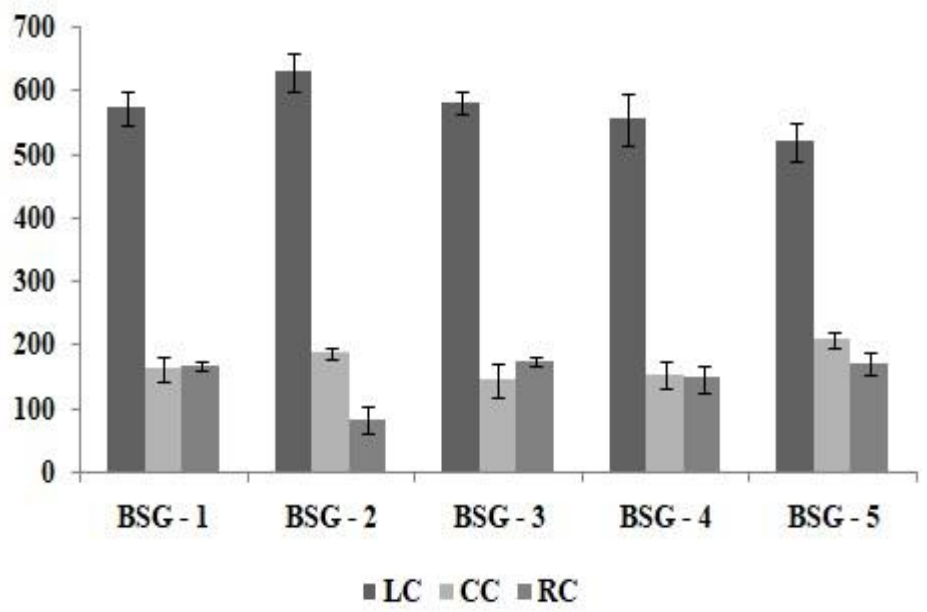

Figure 6: Behavioral responses of the SPBS (secondary phase of behavioral studies) testing phase showed the antibiotic oral infusions (AOIs) had a greater impact on the retrieval of learned information in the infusive groups (BSG -2, 3, 4 and 5) compared to the non-infusive group (BSG -1). The number of attempts was reduced to the positive reward chamber (right chamber - RC) compared to the negative reward chamber (left chamber-LC) which shows the impact of AOIs

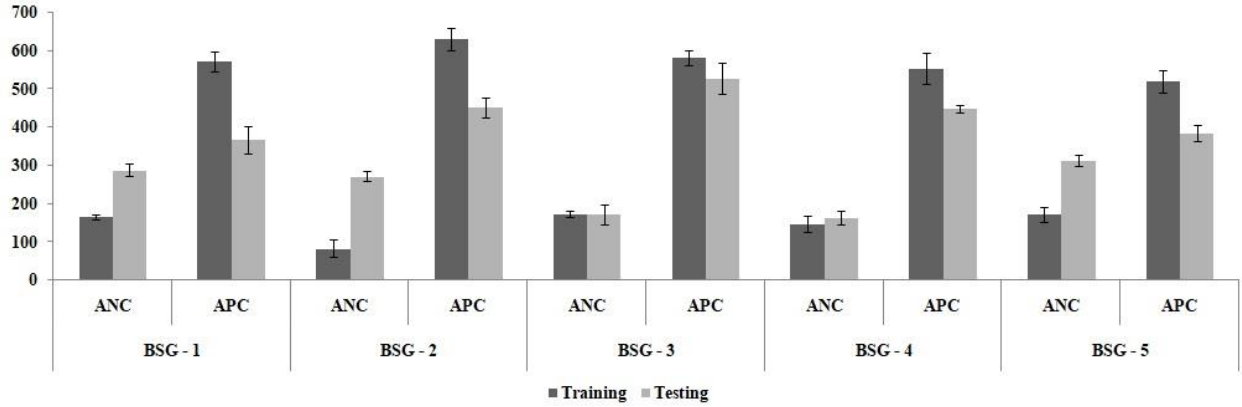

Figure 7: Comparative analysis of SPBS (secondary phase of behavioral studies) training and testing phases showed that AOI (antibiotic oral infusions) may have a mild/null impact on the learning abilities of the infusive BSGs (BSG - 2, 3, 4 and 5) compared to non-infusive BSG (BSG -1). Behavioral responses showed that the reduction in the number of correct choices stated the impact of AOI on the development of cognitive impairment (BSG – Behavioral study group; ANC – Attempts to the negative chamber; APC – Attempts to the positive chamber)

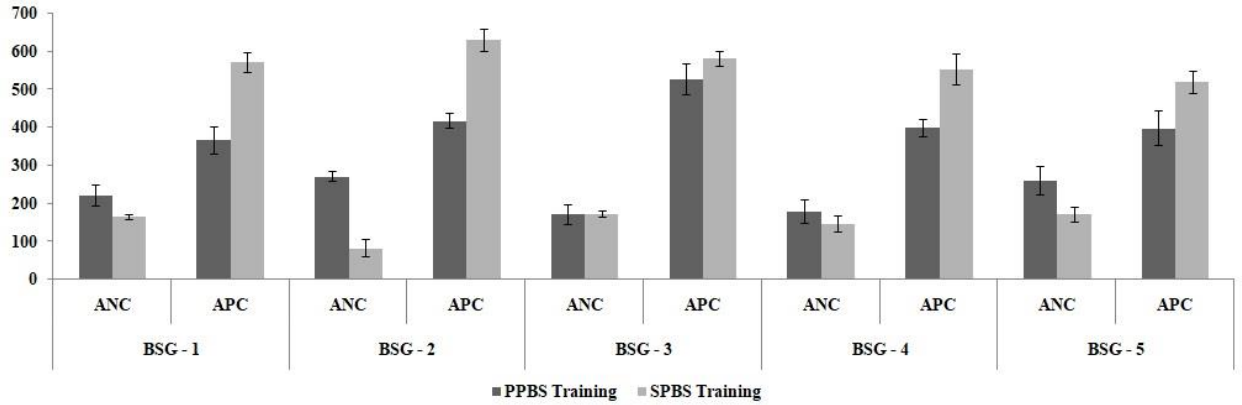

Figure 8: Training responses of the primary phase of behavioral studies (PPBS) and secondary phase of behavioral studies (SPBS) showed that antibiotic gentamicin sulphate did not have any greater effect on the learning abilities of infusive BSGs compared to the non-infusive group (SG - 1) (BSG – Behavioral study group; ANC – Attempts to the negative chamber; APC – Attempts to the positive chamber)

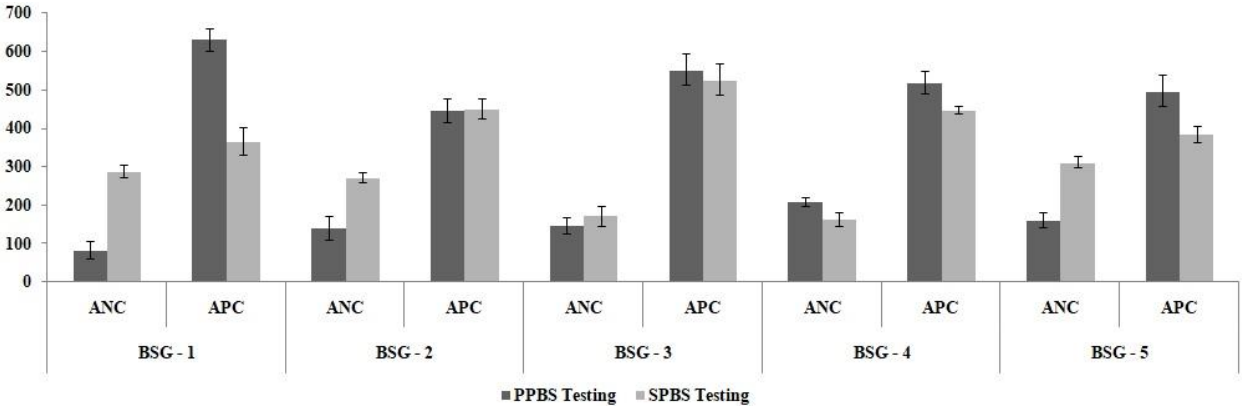

Figure 9: Behavioral testing scores of the primary phase of behavioral studies (PPBS) and secondary phase of behavioral studies (SPBS) phases showed that antibiotic oral infusions (AOI) may have a greater impact on the retrieval of learned information in the infusive BSGs compared to the control (non-infusive BSGs) (BSG – Behavioral study group; ANC – Attempts to the negative chamber; APC – Attempts to the positive chamber)

Impact of Antibiotic Oral Infusions on the Development of Stress Memory Formation: To identify the development of stress in the infusive and non-infusive group after SPBS, two different behavioral tests [open field test (OFT) and predator exposure test (PET)] were carried out between days 30 - 35. Initially, OFT was performed by all five BSGs to identify the development of anxiety-like behavior as a result of AOI. Behavioral responses of infusive BSGs showed that

experimental animals spent more amount of time in the outer compartments compared to the non-infusive BSGs. The obtained experimental results showed that study animals of BSGs – 2, 3, 4 and 5 spent more amount of time in the outer compartment which shows the presence of anxiety-like behavior which is absent in the non-infusive group (BSG - 1).

Thus, the observed result proved that AOI plays a major role in the development of anxiety like behavior in infusive BSGs (Fig. 10). Other than anxiety development, AOI also played a role in the development of fear memory in the BSGs – 2, 3, 4 and 5. Fear memory may develop in the infusive group due to disruptions in the HPA axis or due to imbalanced cortisol production in the BSGs. Behavioral responses of non-infusive and non-infusive BSGs showed that experimental animals that received AOI developed a fear memory during their exposure to the predator on days 33-35. Behavioral scores showed that infusive BSGs spent more amount of time in the complete fear zone (CFZ) compared to the mid-fear zone (MFZ) and the fearless zone (FZ). The observed score was vice versa in the animals of the non-infusive group who spent more amount of time in FZ compared to MFZ and CFZ (Fig. 11).

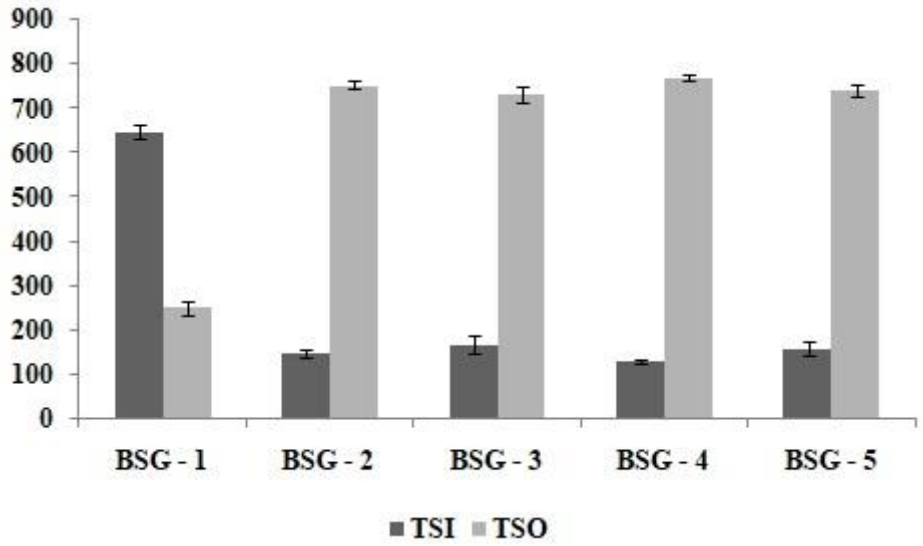

Figure 10: Observed experimental behavioral results of open field test (OFT) showed that study animals of BSGs – 2, 3, 4 and 5 spent more amount of time in the outer compartment compared to the inner compartment which shows the presence of anxiety-like behavior in infusive groups compared to the non-infusive group (BSG -1) (BSG – Behavioral study group; TSI –time spent in the inner compartment; TSO –time spent in the outer compartment)

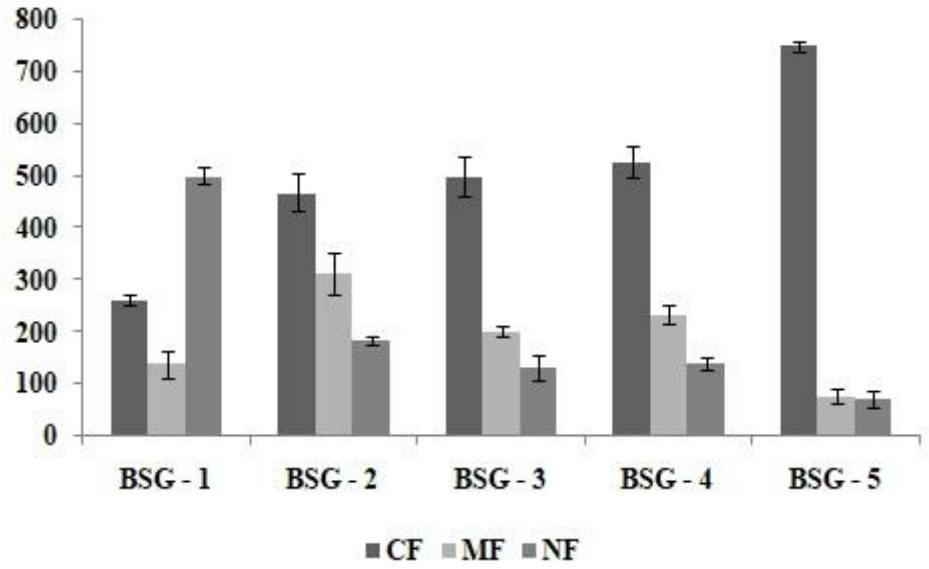

Figure 11: Observed behavioral scores of the predator exposure test (PET) showed that infusive Behavioral study groups (BSGs) spent more amount of time in the complete fear zone (CFZ) compared to the mid-fear zone (MFZ) and the fearless zone (FZ). Thus, the plotted bar diagram showed the effect of antibiotic oral infusions (AOIs) on the development of fear memory compared to non-infusive group

Discussion

In a stress-free environment, CREB- mediated neuronal signaling pathway is activated by the activation and phosphorylation of specific neuronal molecules like adenyl cyclase (AC), cAMP response element (cAMP), protein kinase – A (PKA) and cAMP response element binding protein (CREB)[11,12,29,31,40,51,52]. Later on, phosphorylation of CREB results in the activation of immediate early gene (IEG) cascades. Activation of IEG cascades results in the induction of memory formation through the activity-depended expression of microRNA - 132 (MiR – 132) which regulates the production of post-synaptic density proteins (PSDs) like calcium/calmodulin-stimulated protein kinase (CaMK-II) and post-synaptic density protein (PSD-95)[24,32,33].

Combined expression of these neuronal signaling molecules results in the development of long-lasting cognitive memory in a particular host system. The formation of cognitive memory results in the storage of learned information in the form of neuronal encrypts (NEs). These NEs may result in the maintenance of various homeostasis mechanisms within the host[14,30,45]. The formed homeostatic state can be disturbed by the continuous/ higher intake of antibiotics for a limited period. Intake of antibiotics results in the formation of oral/gut dysbiosis. Formation of oral/gut dysbiosis results in aberration or a decrease in the beneficial microflora present throughout the GI tract[6,18,41,42].

The present study made an initial attempt to show the impact of oral antibiotic intake in the formation of cognitive impairment through oral/gut dysbiosis. Recent research findings showed that the residential flora of the oral cavity and gut play a major role in the development of cognitive memory formation through the production and transmission of NPs from the gut to the brain through the BBB. Transmitted NPs were responsible for the production of specific neurotransmitters within the PSN. Further, produced neurotransmitters may result in the formation of cognitive memory through the CREB-mediated neuronal signaling pathway in a healthy state[3,26,29,35,43].

During disease conditions, pathogenic colonization may result in microbiota imbalance in the oral cavity and gut. This microbiota imbalance may be caused by the emergence of AMR which reduces beneficial microflora in the GI tract. These stated conditions result in the formation of oral/gut dysbiosis [2,22,26,39]. This dysbiosed state results in the reduced synthesis of NPs from the gut and also causes inflammation in the gut. Formed gut inflammation further results in the reduced transmission of least available NPs to the brain and forms cognitive impairment [2,26,37,38]. The present study also reported that other than oral/gut dysbiosis antibiotic intake may also be responsible for the development of cognitive decline.

According to recent reports, antibiotic intake during early life may have a greater impact on the gut microbiome and its associated metabolic effects. Other than metabolic defects, it also causes disturbances in the normal GI microbiota of a host. These disturbances result in the development of cognitive impairment through the imbalances in the formation of NPs within the gut[36,42,50]. Further, gut microbiota imbalance results in the development of cognitive decline according to the level of antibiotic intake and development of AMR within the host system[20,30,36]. Thus the present study proved that antibiotic intake had a severe effect on the development of oral/gut dysbiosis which results in the development of decreased/impaired cognitive functions.

Conclusion

The present study tried to elucidate the role of antibiotic oral infusions (gentamicin sulphate) in the development of cognitive impairment through oral/gut dysbiosis. During disease conditions, antibiotic intake may aberrate the native microflora of the GI tract by increasing the pathogenic microorganisms within the oral cavity and gut. Thereby, it results in the formation of oral and gut dysbiosis. Formed dysbiosed state results in the production of reduced NPs within the gut and also results in the causation of inflammation in the BBB. Formed inflammation results in the reduced transmission of the least available NPs from the gut to the brain.

Reduced transmission results in the formation of impaired cognitive memory in different brain regions responsible for memory consolidation. The outcome of the present study showed the effect of antibiotics in the learning of new information and retrieval of learned information through the process of SPBS training and testing. Observed experimental results also state the hindrance effect of antibiotics in the formation of cognitive functions within the brain. Thus, the present study laid a path for identifying the effect of antimicrobial resistance and its effect on oral/gut dysbiosis in our future studies.

Acknowledgement

MM expresses sincere thanks to the Department of Science and Technology (DST), Government of India (GoI) for the establishment of the DST-FIST facility for Genomics and Proteomics in the Department of Biotechnology, Sri Ramakrishna College of Arts & Science (Autonomous), Coimbatore – 641 006, Tamil Nadu, India under the DST-FIST PG College Level – A Program (SR/FST/COLLEGE-/2022/1203).

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(Received 16th March 2024, accepted 24th May 2024)