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Research Article

Motor and Cognitive Outcomes of Environmental Enrichment during Development in Male BALB/c mice.

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ABSTRACT

Introduction: Environmental enrichment has been shown to modify synaptic plasticity and change neuronal network structure to improve or preserve function in some rodent models.

Objective: Nonetheless, the unique neurobehavioural implication of enrichment in the BALB/c mice during early development has been sparsely documented.

Methods: Twelve male BALB/c mice were divided into two groups after weaning into separate but similar cages with enrichment using toys and without enrichment for eight weeks. Using the ANY-Maze behavioural tracking software, we tracked behaviour in the Open Field Task and the Novel Object Recognition Task (NORT).

Results: Our findings showed significant improvement in cognitive and motor function with environmental enrichment. We observed increased speed, distance covered, number of line crossings and exploratory behaviour with environmental enrichment. Importantly, we identified a difference in the discrimination index and the percentage time of investigation, implying an increased orientation toward the novel object in the enriched group in the NORT Task.

Conclusion: These findings suggest that environmental enrichment improves cognitive and motor function in the course of development in the BALB/c mice and may be a beneficial strategy for improving neuronal function in health, ageing or disease.

INTRODUCTION

Previously, there have been evidences of changes in neuronal architecture and structural plasticity represented by changes in dendritic spines density with environmental enrichment in rodent models.[1-3] Environmental enrichment refers to the sensory, motor and cognitive stimulation that is specific for a specie which is achieved by simulating the natural environment in the best possible way such that the species specific behaviour is preserved and the wellbeing of the animal is not compromised.[4,5] Without environmental adjustments, experimental animals kept in a "standard" laboratory cages may suffer induced stress which may alter their behavioural phenotypes, cause genetic changes and impair function. Over the years of much experimentation on environmental enrichment, a number of laboratories around the world have put to use such information on environmental enrichment for experimental animals which favour their natural behavioural tendencies.[6] For example, some research institutions or animal facilities have incorporated placing cotton pads in the home cages of rodents to allow for nesting behaviour. Although the influence of enriched environment in a number of rodent models have been studied[7], there are currently sparse data on the neurobehavioural consequences of enrichment in the BALB/c mice during development. The BALB/c mice is largely used in many laboratories around the world and locally in Nigeria. A justification to carry out this investigation is that while animals in the wild are usually exposed to various environmental stimulation through objects, play or nesting material, the opposite is seen in animal facilities where animals are confined to a limited space with just food and water with little or no environmental enrichment. Non-enrichment may cause epigenetic changes with transgenerational outcomes in neuronal or synaptic function in a particular specie.[8] Furthermore, the behavioural outcomes of environmental stimulation can include critical periods during development as well as adulthood or aged.[9,10] The changes are largely not due to changes in DNA sequence, but are phenotypically acquired due to environmental stimulation which impacts gene expression.[11] Further evidences have shown that environmental enrichment may be able to initiate neurogenesis and spine density.[1] The new neurons formed are believed to contribute to the improved cognitive function.[12-14] In this study, we carried out investigations on motor and cognitive functions in enriched versus non-

enriched male BALB/c mice during early development using the open field and novel object recognition methods of assessing function. Our findings showed improved locomotor activity, exploratory behaviour, learning and memory in mice raised in an enriched environment.

MATERIALS AND METHODS

We assessed locomotor activity (motor function), exploratory behaviour, learning and memory in BALB/c comprising twelve (12) randomly divided into two groups after weaning at three to four weeks old. The twelve male BALB/c mice were bred and nursed in the Animal House, Lagos State University College of Medicine. Mice were divided into two groups which include the control mice raised in standard environment with no toys, (n=5) and those raised in an enriched environment (n=7), with plastic coloured toys. Both groups were provided with food and water *ad libitum*, while beddings were changed at least once weekly. The shapes of the toys in the enriched cage were cleaned and changed every week to expose enriched mice to variety of shapes. After weaning, mice were nursed in the experimental cages (standard environment or enriched environment) for eight (8) weeks. Experiments were carried out after eight (8) weeks and were conducted in accordance with the Animal Ethics and Guidelines of the Lagos State University College of Medicine approved by the Animal Ethics Committee.

Open Field – (Assessment of Locomotor Activity and Exploratory Behaviour)

Locomotor activity and explorative behaviour experiments were conducted in an Open Field (OF) box (40cm x 40 cm). The floor of the OF was made from fine polished wood. The four walls were made of clear glass so that the mouse can be observed from the sides of the apparatus as well as from the open top. A camera (Logitech C270, UK), connected to a computer with the ANY-maze Behavioural Tracking Software (Stoelting Co., USA) was placed above the OF apparatus. Using the ANY-maze Behavioural Tracking Software, the boundaries and dimensions of the box were defined. Using the ANY-Maze software protocol, lines on the floor of the OF box were divided into sixteen 10cm x 10 cm squares, and these lines were used to assess activity such as line crossings, time spent in the corners and time spent in the centre zone of the OF box. The centre square (20 x 20 cm) was formed from the four inner (10cm X 10cm) squares and this square was highlighted in the ANY-maze Tracking protocol. Parameter measures for locomotor activity include the speed, total distance travelled within the OF box, and the number of line crossings during the test. Exploratory activity was assessed by the amount of time spent in the center square. The corners were be marked by the 10cm X 10 cm squares at the four corners of the OF box.

Novel object Recognition Test (Assessment of Learning and Memory Function

The Novel Object Recognition Task (NORT) which was initially developed for rats as a test of declarative memory by [15], was subsequently validated as a test for declarative memory in mice.[16-18] The Novel Object Recognition Task (NORT) is a preferred method of assessment of learning and memory because it is less stressful for rodents.[18]

The NORT experimental protocol involved an initial habituation for 5 minutes in the apparatus (the same box used for the open field experiment) prior to the NORT, followed by two stages in the novel object recognition task after 24 hours. The first stage was an acquisition trial (Stage 1) followed by the second which was a retention trial (Stage 2). These two stages were separated by a retention period (delay) of 15 minutes. Each mouse is placed in the NORT box and allowed to investigate the identical objects for the duration of 5 minutes in stage 1. After the retention period (15 minutes), stage 2 test was completed. In stage 2, each mouse was presented with a familiar object (one of the objects from stage 1) and a novel object, which was not presented in stage 1. If the animal remembers the familiar object from stage 1, it should spend more time investigating the novel object during stage 2. The novel object in stage 2 was the same for all the mice tested in the NORT experiment.

All mice testing were carried out under diffuse lighting. A video camera (Logitech C270, UK) was suspended above the centre of the box to capture animal activity during the tests. The camera was connected to a computer where behaviour was viewed, scored, recorded, and the data analyzed by the ANY-maze Behavioural Tracking System (Stoelting Company, USA) software.

Mice were carried to the test room in their home cages and tested for the behavioural tasks individually. Each mouse was moved from their home cage to the testing apparatus using a small platform that the mouse can comfortably rest on. After each 5 minutes' test, each mouse was returned to the home cage and the OF apparatus was cleaned with 70% ethanol (ethyl alcohol) and allowed to dry between trials which removed any olfactory cues in the test box. This procedure was used for all the mice tested. The behavioural measures scored during the NORT [16,17] include:

Line Crossing: The frequency with which the mouse crossed from the square divisions in the open field box

Rearing: The frequency with which the mouse stood on their hind feet or against the wall in any part of the box

Grooming: The frequency and duration of time each mouse spent licking or scratching itself while stationary.

Time of orientation towards the novel object: **Percentage investigation time** of the familiar and novel object for each mouse: calculated as Time animal was oriented towards the novel object x 100 / Time animal was oriented towards novel object + Time animal was oriented towards familiar object.[24]

The Discrimination Index calculated as Time animal was oriented towards novel object – Time animal was oriented towards familiar object / Time animal was oriented towards the novel object + Time animal was oriented towards familiar object.[24]

Data and Statistical Analysis

Data generated is expressed as ±Standard Error of Mean and analysed. The ANY-maze Behavioural Tracking Software completed statistical analysis using appropriate test from data generated (unpaired t-test). Statistical significance was set at $p \leq 0.05$ for each parameter measured.

RESULTS

Motor Function Studies (Locomotor Function)

The motor activity measures studied were total distance travelled in the OF box, the distance travelled in the centre (zone) of the OF box, the total number of line crossings in the centre of the OF box and the total time spent in the centre of the OF box. The centre zone was marked by the four centre squares measuring 10 cm X 10 cm in the OF box. All motor function assessment showed significant increase in the environmental enriched BALB/c mice compared with standard environment raised mice, $p \leq 0.05$. Exploratory

Exploratory Behaviour

Exploratory activity measures in standard and enriched environment raised BALB/c mice showed significantly increased rearing in the enriched group. The total number of rearing episodes, the total time of rearing, the number of rearing episodes at the corners and time spent rearing at the corners increased significantly with enrichment. Additionally, time spent grooming at the corners and the number of grooming episodes which are indices for non-exploration was reduced in the enriched group, although the values were not significant.

Learning and Memory

The results of the Novel Object Recognition Task (NORT) experiments showed a significantly increased cognitive function (learning and memory) in the environmental enriched group compared with the standard group. Importantly the results showed increased distance travelled, speed, line crossings, number of rearing episodes and the time spent rearing in the stage 2 of the NORT (the stage when the novel object was introduced. Additionally, the number of orientation towards the novel object was significantly raised in the enriched group.

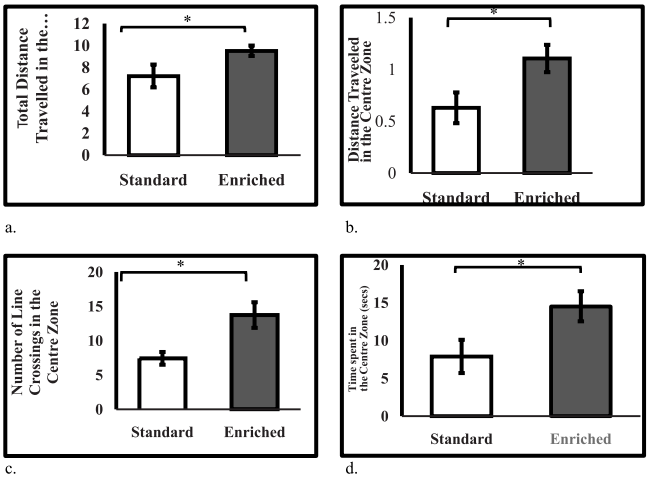

Figure 1(a-d): Total distance travelled in the OF box for a period of 300 seconds (5 minutes) in standard versus enriched environment mice (a) Enriched mice covered significantly longer distance during the test time, *$p \leq 0.05$; (b) The distance travelled in the centre zone of the OF Box in 300 seconds was significantly increased in mice raised in enriched cages, *$p \leq 0.05$; (c) The number of line crossings in the centre zone, an index of locomotor function, was significantly increased in the environmental enriched group, *$p \leq 0.05$; (d) The time spent in the centre of the OF box increased with environmental enrichment, although both groups spent more time outside the centre, $p \leq 0.05$.

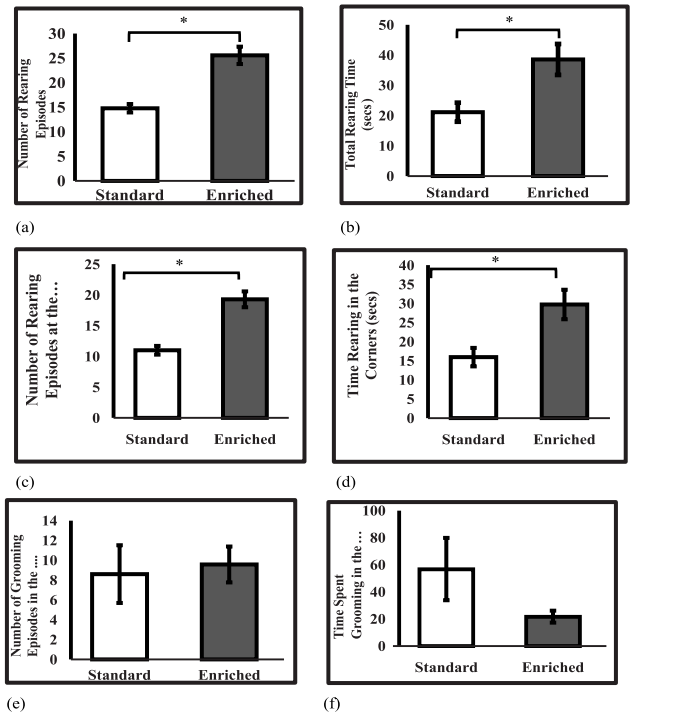

Figure 2 (a-f): (a) The number of rearing episodes an index of exploratory function increased significantly in the enriched group for the period of the test, 300 seconds, *$p \leq 0.05$ (b) The total time spent rearing which is an index of exploratory function was significantly raised in the enriched group for the period of the test, 300 seconds, *$p \leq 0.05$ (c) The number of rearing episodes at the corners showed significant increase in the enriched group showing that activity at the corners were more exploratory, *$p \leq 0.05$ (d) The time spent rearing at the corners were considerably lower than the entire time spent in the OF box for both groups in 300 seconds, however the time spent rearing at the corners was significantly raised in the enriched group, *$p \leq 0.05$; (e and f) Grooming activity was generally lower in the enriched group compared with the standard group, although the values were not significant.

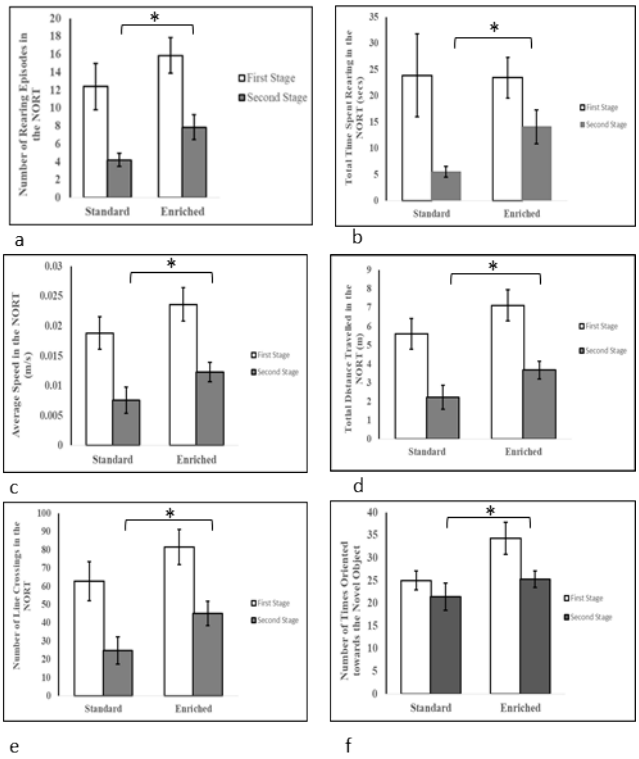

Figure 3 (a-f): (a) The number of rearing episodes in the second stage of the NORT experiment increased significantly in the enriched group, *p≤0.05 (b) The total time spent rearing increased significantly in the NORT in the enriched group compared with mice raised in standard environment, *p≤0.05 (c) The average speed in the NORT was significantly raised, in the second stage, in the enriched group showing an increased response to environmental stimulation with the novel object, *p≤0.05; (d) The total distance travelled increased in the enriched group in the second stage of the NORT when a novel object was introduced, *p≤0.05; (e) The number of line crossings in the NORT increased significantly in the enriched group, the increased line crossings which is an index of exploratory activity occurred in the second stage of the NORT when a novel object was introduced, *p≤0.05; (f) the number of times of orientation towards the novel object was significantly raised in the enriched mice, *p≤0.05.

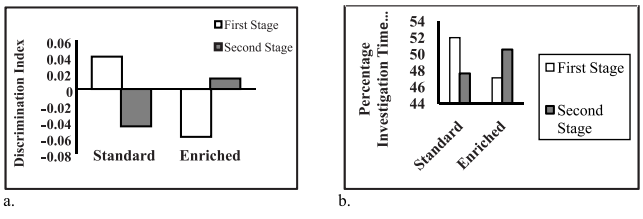

Figure 4a, 4b: (a) the discrimination index refers to the calculated value for the measure of differentiating the novel object from the familiar object, a positive value shows more time investigating the novel object (b) the percentage investigation time measures the amount of time spent investigating the novel object compared with the familiar object, a percentage investigation time of above 50% shows that mice in the group spent more time investigating the novel object in stage 2 of the NORT. Enriched environment raised mice showed a percentage investigation time of 55.55% while standard environment raised mice had a percentage investigation time of of 47.11%.

DISCUSSION

Motor and Locomotor Function

The BALB/c mice showed improved motor function (locomotor function) with environmental enrichment during early development. For the enriched mice, it may be reasonable to attribute the improved function to exercise as the toys presented in the cage of enriched mice may be seen as exercise tools. Nevertheless, exercise only may not be the basis for improved function, as both exercise and enriched environmental stimulation have been shown to separately improve function. Visual stimulation presented early in life may play an important role in this improvement observed as earlier reported.[19,20] The distinction between enrichment and exercise in this study was substantiated by the use of colored toys which are visually stimulating as opposed to some other studies where the running wheel (an exercise object) was used as enrichment in the animal cage. Herein, we suggest that the improved motor function observed in this study may be linked to enrichment by visual stimulation rather than by exercise. In the future, it is recommended that experiments be designed in such a way that protocols clearly separate the possible influence of exercise from sensory stimulation. Notwithstanding, depending on the environmental stimulation chosen during experimentation, behavioural outcomes may occur as a result of either enrichment or exercise or both, which are beneficial for motor function.

Exploratory behaviour

As a measure of exploration, the speed and distance travelled was increased in the enriched group compared with mice in the standard group. Although all mice groom, excessive grooming has been implicated in obsessive compulsive disorder.[21] First, our findings revealed that grooming occurred for less than 20% of the total test time in both groups, which is within the limits for normal grooming in rodents.[21,22] Regardless of this observation, grooming time was more in mice raised in standard environment, although it was not significantly increased. Mice used in this study were approximately three months old at the termination of the experiments, hence we speculate that due to changes that may occur in brain structure and function with age, grooming may increase as mice grow older in a standard environment. This implies that with increasing age grooming time and frequency may increase significantly in older mice. Additionally, we found that rearing episodes and time spent rearing were significantly increased in enriched environment raised mice. Rearing is considered an exploratory activity and characteristic of reduced levels of anxiety.[23]

Learning and Memory

Our results showed that there was an improvement in learning and memory in the NORT. Herein, we report a positive value for the discrimination index (a positive value means more time investigating the novel object) and percentage investigation time greater than 50% (>50% is considered as greater investigation of the novel object or location) in the enriched group. These findings have been compared with standardized testing outcomes for the NORT

reported by [24], that a positive discrimination index and a percentage investigation time greater than 50% imply better memory function. Furthermore, we observed that the minimum distance from the novel object in the second stage of the NORT was shorter in the enriched group compared with the standard group. A significant difference in the minimum distance from the novel object has been previously reported in old rats compared with young [25] as a measure of memory function. This reveals that the enriched mice had a closer interaction with the novel object than mice raised in standard cages.

CONCLUSION

Consequently, we conclude that during early development in the BALB/c mice, environmental enrichment improved cognitive and motor function. Our findings further provide supporting evidence on the impact environmental conditions may have on experimental data and outcomes when using the BALB/c mice. This study also open new lines of enquiry into identifying the synaptic plasticity properties (Long Term Potentiation and Long Term Depression), cellular, molecular and epigenetic basis for our findings. Finally, we anticipate that in the near future, enriched environment could be a potential therapeutic tool which can be used to improve function or treat cognitive and motor function disorders in clinical care.

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