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Chapter

Pyramid Exploration Intervention, Environmental Enrichment, Aerobic Swimming Exercise and Brain Neuroplasticity in the Kainate Rat Model of Temporal Lobe Epilepsy

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Abstract

Previous studies have shown that environmental enrichment increases neurogenesis and reverses learning and memory deficits in rats with kainate-induced seizures. We tested the hypothesis that exploring a wooden pyramid for 3h/d augments neurogenesis and attenuates the learning and memory deficits following chemical lesioning of the hippocampus and motor cortex with kainic acid (KA). A pyramid exploration intervention (PEI) was created by subjecting rats to residing in a pyramidal wooden structure of 3 h/d for 30 d. We also compared the effects on neurogenesis for PEI to those for aerobic (swimming) exercise (EX) and environmental enrichment via exploration of a rectangular-shaped wooden cage. Following KA seizures, the PEI increased brain neurogenesis. Differences in measures of neurogenesis were not significantly different than those for EX and EE. Aerobic (swimming) exercise and novel environment exposures appear to increase neural plasticity and may be considered a complementary treatment for epilepsy.

Keywords: neurogenesis, neural plasticity, environment, learning, memory, behavior, amygdala, hippocampus, motor cortex, complementary and alternative medicine

1. Introduction

Previous studies from our laboratory have shown that regimens of short periods of daily swimming exercise and environmental enrichment increases brain neurogenesis, learning and memory in the kainate rat model of temporal lobe epilepsy [1–3]. Temporal lobe epilepsy (TLE) is associated with oxidative stress, a putative mechanism for neuronal apoptosis [1] and decrements in neural plasticity that impairs the brain’s cognitive functions [2]. Oxidative stress is known to reduce neuronal antioxidant and anti-apoptotic activity which damages the brain's learning and memory networks [3]. Epilepsy is often resistant
to treatment with antiepileptic drugs that are also known to produce oxidative stress and to promote neuronal apoptosis [4]. Manipulation of an animal’s environment appears to have the potential for producing physiological, therapeutic effects and are purported to function as environmental enrichment schemes for improving responsiveness to drug treatments. We have reported increases in neurogenesis and improvements in performance of rats on learning and memory tasks associated with environmental enrichment by allowing rats to explore a novel cage with different objects in different configurations for 3 h/d, for 30 d. Another such environmental enrichment intervention involves subjecting rats to stress restraint in a pyramidal wooden structure. This environmental intervention is shown to reduce the physiological and oxidative stresses associated with immobilization and restraint [5].

Nikola Tesla was a mathematician-physicist and the inventor of many of today’s most advanced electromagnetic technologies [6]. Among Tesla’s hypotheses more, than a hundred years ago, is the prediction that the architectural design of the great pyramid of Egypt produces electromagnetic effects within its chambers [7]. Pyramids are reported to augment local electric fields via a lightning-rood effect [8] and function as electromagnetic conductors [7]; although, the mechanisms remain unclear. It may, therefore, not be an accident of nature that pyramidal-shaped neurons evolved to generate and focus electromagnetic energy in the brain. Pyramidal-shaped neurons comprise 12 billion of the 16 billion neurons in the human cerebral cortex [9]. A psychiatrist-inventor, Hans Berger, in the 1920s, was the first person to demonstrate electroencephalographically-measured brain waves (EEG) [10]. It is now known that each brain wave represents the synchronized action potentials of millions of pyramidal cells in the cerebral cortex [11]. In view of these interesting aspects of pyramids and pyramidal cells in the brain, the present study is designed to test the hypothesis that a pyramid exploration intervention (PEI) augments brain neurogenesis and improves the brain’s learning and memory functions in an animal model of TLE.

2. Methods

These studies were approved by the institutional animal care and use review board of Manipal University, Bangalore India, in partnership with the American University of Antigua College of Medicine.

2.1 Animals

The animal subjects were 4-month-old male Wistar rats maintained under conditions of 12-hour light-dark diurnal cycles in Manipal University’s Animal Research Facility. The animals were fed ad libitum with a normal balanced rat chow diet.

2.2 Experimental design

Figure 1 depicts the study design intended to determine the effects of a pyramid exploration intervention (PEI) on neurogenesis, learning and memory functions in rats. The animals were grouped as follows: 1. Normal control rats (NC); 2. Normal control rats with exposure to the PEI (NC + PEI); 3. Sham control rats with exposure to the PEI (SC + PEI); 4. Kainic acid-lesioned rats; and 5. Kainic acid lesioned rats with exposure to the PEI (KA + PEI). NC animals were undisturbed in their home cages. NC + PEI animals were subjected to the PEI for 3 h/d. SC + PEI
animals were subjected to sham surgery followed by exposure to the PEI. The sham surgery involved fixing the rats in a stereotaxic apparatus under general anesthesia after which burr holes were drilled in the skull using stereotaxic coordinates for the lateral ventricles taken from a standard rat brain atlas. A Hamilton syringe was inserted into the lateral ventricles bilaterally and removed without administration of any fluids or drugs. Following suturing of the scalp wounds, the rats were placed back in their home cages and were subjected to the PEI 3 h/d for 30 d. KA animals were administered kainic acid (KA) bilaterally into the lateral ventricles using a Hamilton syringe. Animals in the KA lesioned + PEI group were administered KA and then underwent PEI for 3 h/d for 30 d. The PEI was initiated 1-d after grouping in the NC animals, 1-d after surgery SC + PEI animals and starting on the first postictal day in the KA + PEI group.

2.3 Surgical and related experimental procedures

SC and KA animals were anesthetized with a mixture of ketamine (50 mg/mL), xylazine (4.5 mg/mL) and acepromazine (0.4 mg/mL) at a dose of 0.70 mL/kg body weight and were fixed in the stereotaxic apparatus with the incisor bar situated 3.7 mm below the inter-aural line. The skull was exposed and a burr hole was drilled guided by the stereotaxic coordinates as follows: 3.7 mm from bregma, 4.1 mm lateral from the midline [12]. KA lesioning was accomplished using a Hamilton syringe needle filled with 0.5 μg/μL KA, 1.0 μl of which was administered slowly over 20 min. The KA-filled syringe was lowered from the stereotaxic syringe holder 4.5 mm to reach the lateral ventricles. After the needle was withdrawn, the skin was sutured and the animals were returned to their home cages.

2.4 Pyramid exploration intervention (PEI)

Exposure of animals to PEI was accomplished in a wooden cage of larger dimension than the steel home cage (Figure 2). Rats were allowed to explore the PEI environment for 3 h every d for 30 d, beginning immediately following either grouping (normal control group), sham operation (sham-operated control group) or KA + lesioning (kainate experimental group) for 3 h/d for 30 d beginning 1-d the grouping (normal controls), sham operation (sham controls) or KA lesioning.
2.5 Morphological procedures

Identification of surviving neurons. Surviving neurons were identified by cresyl violet staining of neuronal Nissl substance. Rats were deeply anesthetized with ether and fixation was performed by transcardial perfusion of the left ventricle with 15 ml of 0.9% heparinized saline at 1 mL/min, followed by perfusion with approximately 250 mL of 10% formalin at 1 mL/min. Brains were excised following decapitation. Coronal sections (5-6 mm thickness) were cut and were post-fixed for 24 h using 10% formalin. Tissues were then dehydrated in 70% alcohol for 2 h, 90% alcohol for 2 h, 3 changes in 100% alcohol for 2 h, clearing with xylene for 2 h and embedded in paraffin. A rotatory microtome was used to cut 5 μm thick sections from the mid-dorsal hippocampus and motor cortex. Sections were then mounted serially on gelatinized slides and stained with 0.1% cresyl violet at pH 3.5-3.8. Cresyl violet staining was followed by sequential treatment with 90% and 100% alcohol for 1-2 minutes each, xylene for 2 minutes, followed by mounting in DPX.

Cell counting. Surviving neurons were counted using light microscopy. Total number of surviving neurons were counted in 10 randomly-selected fields, at 40× magnification (Magnus, Olympus Pvt. Ltd. New Delhi, India) and were averaged. Cells with pyknotic nuclei were excluded from the count. The researcher doing the counting was blinded to the animal grouping and experimental treatment.

Identification of dendritic branch points and intersections. Dendritic branch points and intersection were identified by the Golgi-Cox staining procedure with some modifications [13]. Using the same procedure as described above for anesthetization, the brains were quickly removed and incubated in Golgi-Cox fixative, without perfusion or post-fixation. Tissue collected from individual animals were fixed in individual bottles as follows: Brains were maintained as fresh as possible, placed in clean bottles on glass wool or gauze, covered with Golgi-Cox solution and left at room temperature in a room without light to limit oxidation. After 2 days, the Golgi-Cox solution was changed. The brains were exposed to the fixative for 2 weeks followed by impregnation in Golgi-Cox solution and dehydration in the following order: 50% ethanol and 70% ethanol for 1 hour each, 90% ethanol for 2 hours, 100% ethanol for 1 hour. The tissue blocks were then blotted to remove the alcohol from their surface, after which they were carefully mounted on a tissue holder by applying 2 drops of Fevikwik adhesive on the wooden block and the tissue was fixed. Sections were then cut using a base sledge microtome to a thickness
of 120 μm. Using a soft brush, sections were collected in 70% ethanol, washed in distilled water for 5 minutes, 5% sodium carbonate for 20 minutes, distilled water for 5 minutes, 70% ethanol–10 minutes consisting of 2 washes for 5 minutes each, 90% ethanol for 10 minutes consisting of 2 washes for 5 minutes each, 100% ethanol for 10 minutes consisting of 2 washes for 5 minutes each, Cedar wood oil for 1 hour, xylene for 10 minutes consisting of 2 washes for 5 minutes each. Sections were mounted on a glass slide using DPX.

**Counting of dendritic branch points and intersections.** The dendritic branch points and intersections of darkly-stained neurons throughout their arborizations were counted using camera lucida tracing equipment (Dutta Scientific, Bangalore). Neurons exhibiting truncated dendritic branches within a 100 μm radius of the soma were excluded. Interference from adjacent neurons was eliminated as described for neuronal counts. Counting of dendritic branch points and intersections was accomplished by Sholl’s concentric circle method [14]. Concentric circles were drawn on a transparent sheet with 20 μm as the radial distance between two adjacent circles. The concentric circles template was placed on the camera lucida-traced neuron so that the center of the neuron’s soma coincided with the center of a circle. Then, the number of branch points between the two adjacent circles were counted. Intersections were defined as points of dendrite touching or intersecting with a circle. Branch points and intersections were counted up to a radial distance of 100 μm from the neuronal cell body.

**2.6 Behavioral testing**

Behavioral tests were administered on the 42nd day following grouping for the NC group and on the 42nd day following surgery or KA-induced seizures for the other groups. All testing was done at approximately 7:00 PM to control for the diurnal variation of night-time activity in rodents.

**The T–maze task.** Rats were subjected to left-right discrimination, a spatial memory task testing the animal’s ability to discriminate the left or right arm of a T-maze for a food reward. The animals were food-deprived for 2 days prior to testing to enhance motivation. The rats were subjected to an orientation period by being placed in the start box for 60 s. The animals were then permitted to explore the T–maze for 30 minutes and to ingest 15 pellets (10 mg/pellet) in each goal area. The animals were then returned to the start box. The orientation was done for 2 consecutive days followed by 6 trials/day for 4 consecutive days.

**Spontaneous alternation test.** Each animal, after being placed in the start box, was allowed to move into the maze structure where they selected one of the branches of the maze, and once they ingested a food pellet in the goal area they were placed back in the start box for the next trial. The interval between trials was one minute and the maze branch selected by the rat was recorded. At the end of the 4-day experimental period, the total number of branch alternations was used to compute percent bias as follows: (Number of selections of most frequently selected branch ÷ Number of trials) x 100.

**Rewarded alternation test.** One day after completing the spontaneous alternation test described above, six trials of the rewarded alternation test were performed daily. Each trial included two runs consisting of a forced and a selection run. For the forced run, the animals were forced into one of the branches of the maze by blocking the other branch and they were permitted to ingest the food pellets within the goal area. After eating the food in the goal area, they were placed back in the start box to perform the selection run. For the selection run, the goal area of the forced branch of the maze was kept empty and food pellets were placed in the goal area of the alternate branch. Both maze branches were accessible to the animals;
a one-minute pause separated each forced and selection run and there was another one-minute pause between each trial. Maze branch selection was predetermined and was the same for all animals on a given day; on the next day, the forced branch was changed. For the selection run, a correct response was recorded whenever the branch opposite to the forced branch was selected and vice versa for recording of an incorrect response. Percentage of correct responses was computed as follows: 
(Number of correct responses ÷ Number of trials) x 100. 

**Passive-avoidance learning and memory test.** This test has the following 3 parts: i) an exploration test, ii) an aversive stimulation and learning phase (passive-avoidance acquisition), and iii) a retention test. 

**Exploration test.** Each animal performed three exploration tests per day. The interval between trials was five minutes and each trial was three minutes in duration. Each animal was placed in the center of a large compartment facing away from the entrance to a dark small one. The door between the large and small compartments was open and the animal was permitted to explore both the large and small compartments for three minutes. Time within the large compartment, time within the small compartment and number of crossings from large to small compartments were recorded as a measure of exploration. Animals were then replaced in the home cage, where they were maintained for the five-minute interval between trials and this sequence was repeated three thrice for each animal. 

**Aversive stimulation and learning phase: passive-avoidance acquisition test.** Each animal was forced into the smaller compartment and the sliding door between the two compartments was closed following the last exploration trial. Then, 3 strong electric foot pulses of 50 Hertz, 1.5 milliamps, 1.0 second in duration were administered at five-second intervals, and the animal was returned to their home cage. 

**Memory retention task.** The memory retention task was done twenty-four hours after the previously described acquisition test. For this test, the animals were maintained in the center of the large compartment and each animal was permitted to explore the compartments for three minutes after which they were returned to their home cages. This sequence was repeated three times with an interval between trials of five minutes. Time in the large compartment, time in the small compartment and number of crossings from large to small compartment were recorded as measures of exploration. 

2.7 Data analysis 

Analysis of variance (ANOVA) with Bonferroni's post-hoc test was performed to estimate the statistical significance of differences between groups (GraphPad Prism, version 5). Across the groups, correlations between the most relevant morphometric and behavioral measurements were evaluated by Pearson's product moment correlation coefficient (r). Neurogenesis parameters were compared for similar experiments performed under conditions of PEI, aerobic (swimming) exercise and another form of environmental enrichment [1–3]. Statistical significances were guaranteed at $P \leq 0.05$. 

3. Results 

3.1 Morphometric measurements in hippocampus, amygdala and motor cortex 

Figure 3 shows the effects of the PEI, with and without KA lesioning, on the CA3 area of hippocampus by light microscopy. The PEI was associated with
significant increases in the numbers of surviving neurons in the normal and sham-operated control groups and after KA lesioning in the experimental group (data not shown). The PEI also produced significant increases in the numbers of surviving neurons in hippocampal area CA1, dentate gyrus, basolateral amygdala and motor cortex in the controls and in the KA-lesioned animals (data not shown).

Figure 4 depicts the effects of the PEI on the dendritic branching of the surviving neurons in hippocampal area CA3 by light microscopic camera lucida tracings.

Figure 5 shows the morphometric measurements of the effects of the PEI on the counts of branch points and intersections in hippocampal area CA3. These data demonstrate that exposure to the PEI produced significant increases in the branch points and intersections both in the presence and in the absence of KA lesioning. Similar increases were found in dentate gyrus, amygdala and motor cortex and in animals subjected to delayed exposure to the PEI after a delay of 60 d, postictal.

3.2 Behavioral measurements

The PEI was associated with significant increases in the percent bias, percentage of correct responses and the number of alternations on the T-maze learning task.
Percentage of correct responses for the animals subjected to the PEI for 30 d starting at the first postictal day was positively correlated with the number of surviving neurons in all the brain areas studied, across the five study groups ($r = 0.92-0.97$, $P < 0.001$). Percentage of correct responses for these animals was also positively correlated with the number of apical and basal dendritic branch points and intersections in the same brain areas, across the five study groups ($r = 0.88-0.94$, $P < 0.01$).

Figure 6 presents the effects of the PEI on the exploration and retention phases of passive-avoidance testing. The PEI was associated with significant decrements in the time spent within a small compartment where an aversive stimulus was previously administered and in the number of crossings. The same pattern of behavioral responses was observed in animals subjected to the PEI for 30 d immediately, starting 1-d postictal, as well as, after a 60-d delay postictal. Similar to the morphometric and T-maze data, these changes were observed in the animals subjected to the PEI. Time spent in the small compartment avoiding the aversive stimulus was negatively correlated with the number of surviving neurons in all the brain areas studied, across the five study groups ($r = −0.93$ to $−0.99$, $P < 0.001$). Time spent in the small compartment was also negatively correlated with the number of apical and basal dendritic branch points and intersections in the same brain areas, across the five study groups ($r = −0.88$ to $−0.95$, $P < 0.01$).

Table 1 summarizes comparisons of the effects of the pyramid exploration intervention (PEI), environmental enrichment in the absence of pyramid exploration (EE) and aerobic (swimming) exercise (EX) on the numbers of surviving neurons in hippocampal area CA3. There were no significant differences in neurogenesis between these three interventions ($P > 0.1$). Similar results were found for the dendritic branch points and intersections in area CA3 (data not shown).
4. Discussion

The main findings of this study are that morphometrically-measured rat brain neurogenesis and performance-measured learning and memory behavioral tasks are augmented by a pyramidal exploration intervention (PEI). The PEI permitted rats to explore a wooden pyramid for 3 h/d. The PEI was associated with increments in the numbers of surviving neurons, apical and basal dendritic branch...
points and intersections at hippocampal areas CA1 and CA3, amygdala and motor cortex suggestive of increased neurogenesis and improved learning and memory under control and postictal conditions. This evidence of increased neurogenesis is bolstered by highly significant correlations, across the five study groups. We found significant positive correlations between the animals’ percentage of correct responses on a T-maze (learning) task, the numbers of surviving neurons and the numbers of dendritic branch points ($r = 0.88-0.94$). We found similar significant, but negative, correlations between the time the animals spent in a small compartment avoiding an aversive stimulus (memory task) and the numbers of surviving neurons, dendritic branch points and intersections ($r = -0.88$ to $-0.99$). These findings demonstrate that, whether a control or an experimental animal, rats with more neurons and more dendritic intersections (synapses) in the four brain areas studied had greater learning of a T-maze strategy, as well as, greater memory for avoiding an aversive stimulus.

We have previously shown increases in rat brain neurogenesis and improvements in performance on the same learning and memory tasks as employed in the present study associated with an environmental enrichment intervention involving exploration of a wooden square structure containing novel objects in novel configurations for 3 h/d [3]. We designed the present study to expand the previous studies to a different type of environmental intervention, the exploration of a pyramid-shaped wooden house for 3 h/d. We used a pyramid-shaped structure to emulate prior studies demonstrating that housing in pyramid while exposed to restraint stress counters the parameters of oxidative stress associated with the animal restraint [5]. Oxidative stress causes accumulation of superoxide and other free radicals—highly reactive oxygen and nitrogen species such as superoxide and hydroxyl radical anions, nitric oxide, nitrogen dioxide and peroxynitrite [15]. These highly reactive oxygen and nitrogen species (ROS/RNS) do not accumulate under normal conditions because they are neutralized/scavenged by antioxidant enzymes such as superoxide dismutase, catalase and glutathione reductase synthesized by, and stored in, each normal healthy cell [16]. Oxidative stress occurs when the balance between production and neutralizing/scavenging favors accumulation of ROS/RNS [17]. Such accumulation of ROS/RNS causes peroxidation of lipids in the cell’s plasma and organelle membranes [16]. ROS/RNS-dependent enzymes are known to function normally under conditions of very low, physiological levels of ROS/RNS [17].

The aforementioned pyramid exposure appears to protect against the restraint stress-induced atrophy of the hippocampus [5]. Oxidative stress is shown to be a pathophysiologic feature of KA-induced seizures [18]. The PEI in the present study is, like the aforementioned pyramid study involving stress restraint, associated with physiologically-significant effects, evidenced by significant correlations between morphometric and behavioral measurements across the five study groups. Pyramid exposure is also shown to increase the amplitude of electroencephalographically-measured alpha waves [19], and positive mood responses associated with meditation [20, 21]. Electroencephalography and monitoring of emotional functions were beyond the scope of the present study. However, hippocampus is an important source of emotional responsiveness and theta waves [22]. Theta and alpha waves propagate together in the neocortex when cognitive tasks are performed [23]. It is, therefore, plausible that the increases in neurogenesis and improvements in behavior observed in the present study are likely to have been reflected in brain waves and emotional responses.

KA-induced seizures are also a stimulus for neurogenesis, associated with increased numbers of neural progenitor (stem) cells within the subgranular zone of hippocampus, amygdala sensorimotor cortex [24] but motor cortex neurogenesis has not been extensively studied. It was beyond the scope of the present study to
identify neural stem cells and to measure the immediate effect of the KA-induced seizures but we assume that our morphometric counts of surviving neurons, dendritic branch points and intersections after a 30-d period is a fair reflection of the seizure-induced neuronal death followed by neural stem cell proliferation known to occur in the KA rat model of TLE.

5. Conclusion

The results of this study demonstrate that regular exposure of rats to a wooden, pyramidal-shaped environment for their exploration 3 h/d increases neurogenesis learning and memory, under normal control conditions, after a (sham) surgical intervention and following KA-induced seizures. These findings should be interpreted cautiously because previous studies from our laboratory have shown that the same benefits accrue from an environmental enrichment by exposing animals to exploration of a rectangular wooden cage with novel objects in novel configurations for 3 h/d and aerobic (swimming) exercise [1–3]. It is, therefore, likely that pyramid effects are nonspecific and are the result of exposing animals to any novel environment. We, therefore, conclude that pyramid exposure is probably not different than other modes of environmental enrichment for augmenting brain neurogenesis and improving learning and memory functions. Future studies should determine whether exposure to novel environments are effective as complementary or alternative treatments for the wide variety of neurological diseases wherein full recovery of learning and memory functions may be limited by ineffective neurogenesis.

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Conflict of interest statement

The authors report no conflicts-of-interest.

Data availability

The data supporting this research is available upon request to the corresponding author.
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