Proceedings of The National Conference
On Undergraduate Research (NCUR) 2017
University of Memphis, TN
Memphis, Tennessee
April 6-8, 2017

The Nature of Neural Activity in Hippocampus of Rats Evoked by an Object in Place Task

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Abstract

The interaction with novel objects and same-sex conspecifics, known as environmental enrichment (EE), affects neural development, preference, and exploratory behavior. Specifically, EE can impact neural networks associated with spatial memory in adolescent rats. In this study, the influence of EE on neural activation in the hippocampal formation (HF) excitatory pathway was investigated. An object place preference (OPP) task, in which rats interacted with newly placed familiar objects in an open field, was used to stimulate hippocampal activity. A group of non-enriched Long Evans rats (n=14), housed in shoebox cages, underwent an OPP task and 60 minutes of quiet/dark prior to being sacrificed. Enriched rats (n=16), housed similarly, experienced the same task after 18, 90-min EE sessions between postnatal days 25 and 48. Trial 2 of the two-trial OPP task showed how exposure to an environment with consistently and newly placed familiar objects evoked activity within major input and output regions of the HF, including CA1, CA3, dentate gyrus (DG), and subiculum. Brain tissue was processed using immunohistochemistry to visualize c-fos protein expression, a marker of neural activation, and neural densities were quantified via microscopy. Considering the HF as a whole, a history of EE reduced neural activation. In one primary input area, DG (30.9% reduction, $p < 0.09$), and internal processing area CA3 (-18.0%, $p < 0.21$), activity change from controls was not statistically significant. However, in both CA1 (-30.6%, $p < 0.02$) and the subiculum (-34.7%, $p < 0.02$), a primary output zone, EE did reduce neural activation. The results suggest that an EE history suppresses activation as signal moves sequentially throughout the HF. Because EE rats were routinely exposed to novel placement of familiar objects, habituation to such experience may be responsible for the observed forward moving reduction in neural activity in the HF.

Keywords: adolescence, environmental enrichment, novelty preference

1. Introduction

Certain experiences during adolescence may influence an animal's preferences and likelihood to engage in risk-taking behaviors[18]. Such experiences include the interaction with novel objects and same-sex conspecifics, which is often called environmental enrichment (EE) and can impact the brain and behavior of mammals over time[1, 3-8,11-13] . EE is characterized by the opportunity to explore and interact with novel environments, as well as opportunities for physical and social stimulation that are provided in supplement to standard housing conditions[4,20]. Among rats, EE may provide a lasting impact on object recognition, spatial learning and memory, and the neural networks associated with these behaviors[11,17,20]. As an example, EE has been shown to improve performance on spatial learning and memory tasks, as it particularly influences hippocampal neural activity and can enhance neurogenesis in the dentate gyrus region of the hippocampus[13,20,21].

While the outcome of EE is most commonly examined in adults[1,7,19], several researchers have studied EE across the lifespan as well[8,20]. These studies suggest that there may be a developmental period during which EE is most beneficial,

but it has not been specified when this critical period likely occurs[20]. It may be suggested, however, that this period could occur during adolescence, since early exposure to EE can provide a more lasting impact on problem-solving behaviors, as compared to later EE exposure during adulthood[8]. The few studies of enrichment among adolescents also suggest that behavioral effects of EE may differ between adolescent and adult rats[8]. Additionally, adolescence is marked by an increase in risk-taking behaviors, which include increased exploration and preference for novel, potentially threatening aspects of the environment[19]. In rats, exploratory behaviors typically heighten during adolescence, in order to prepare the rat to leave the nest and survive in the wild by seeking potential food sources[10]. Thus, adolescence may serve as an especially relevant timeframe for the study of enrichment effects. For this reason, the current study aimed to assess the impact of EE on neural activation within hippocampal formation (HF) excitatory pathway evoked by an object preference task in adolescent rats.

1.1 Environmental Enrichment

Environmental enrichment (EE) is an experimental paradigm in which rats are able to interact with a variety of objects and same-sex conspecifics, allowing for both physical and social stimulation[1,3-8,10-11]. Exposure to EE conditions characterizes rats as "enriched" relative to those that have not experienced EE. In a typical EE paradigm, objects, some familiar and some new, are reorganized within an environment in novel spatial arrangements on a daily basis, and rats are able to interact with unfamiliar same-sex conspecifics during sessions. Both of these features promote opportunity for novelty exposure and exploration. EE may be studied using various animal models; however, the amount of stimulation during EE sessions must exceed the amount of available stimulation in standard housing conditions, regardless of the subject type[20].

The effects of EE have been widely studied, as enrichment tends to promote significant, beneficial changes to both the brain and behavior of mammals over time[8, 11]. Specially, EE enhances problem solving skills, improves performance on spatial learning and memory tasks, reduces stress, alters emotional responses, and promotes the overall wellbeing of animals[7,20]. As a result, several animal facilities and laboratories have begun to employ enrichment into their standard guidelines of care[4,18].

The behavioral changes that occur in response EE may be explained by global changes that occur in the brain following enrichment, including enhanced synaptogenesis, increased cortical thickness, increased glial density, increased dendritic branching, and enhanced neurogenesis (especially in dentate gyrus, or DG, of the hippocampus)[4,12,20]. EE may also impact levels of certain neurotrophic factors and hypothalamic-pituitary-adrenal (HPA) axis activity, which may promote plasticity and neurogenesis[12,18]. More specifically, EE may enhance levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which promote cell survival and functions associated with synaptic plasticity[4,6,12]. Studies of the HPA axis, which is activated by stress, indicate that EE may also provide an attenuating effect on HPA reactivity[6]. On a short-term basis, EE has been shown to increase neural activation in the hippocampus (especially in DG and *cornu ammonis* 3), indicating that EE may promote changes in the initial response to novelty carried out by these structures[1]. In summary, the effects of EE on neuroanatomical and neurochemical parameters, which in turn, positively impact certain aspects of behavior, may promote the overall wellbeing of enriched rats. In this study, neural activation in the hippocampus, associated with spatial learning and memory, is investigated.

The current study aims to assess the impact of EE on activation in the major excitatory pathway of the hippocampus, known as the hippocampal formation (HF). An object place preference (OPP) task, in which rats interacted with newly placed familiar objects in an open field, was used to stimulate hippocampal activity[2]. This task requires animals to encode, process, and retrieve information about the locations and features of objects in an open field, relying on external environmental cues[2,4] Thus, the OPP task provides an effective paradigm for studying aspects of novelty preference, spatial memory, and exploration in response to EE, which are reliant on processing within the HF.

1.2 Current Investigation

The hippocampus plays a significant role in aspects of learning and memory, including spatial awareness. As a major excitatory pathway of the hippocampus, the HF is involved in several memory processes, such as encoding, consolidation, and retrieval[15]. This pathway receives input from the entorhinal cortex (EC), the major input area of the hippocampus[5,21]. EC projects to DG, which provides input to *cornu ammonis 3* (CA3), then to *cornu ammonis 1* (CA1)[4,5]. DG plays an important role in the encoding of novel spatial relationships. CA1 and CA3 are both involved with the initial formation of spatial memory, as well as the processing and consolidation of novel information[15,21]. Subiculum (Sub) receives input from CA1, and it serves as a major output region of the pathway[4] sending efferents to

the septal complex, nucleus accumbens, anterior thalamus, and mammillary nuclei[9]. Sub also plays an important role in the processing of spatial information[9]. Thus, as a signal progresses through the HF pathway, the four structures of interest are sequentially activated (from DG to CA3 to CA1 to Sub). Activation of this pathway serves as a neural correlate for the processing and encoding of environmental cues, such that activation in the earlier structures of the pathway (i.e. DG and CA3) represent the recognition of novelty, and sequentially later structures of the HF (i.e. CA1 and Sub) represent the processing and encoding of novel information[4,21]. A simplified schematic diagram of the major input and output regions of this HF pathway is shown in Figure 1.

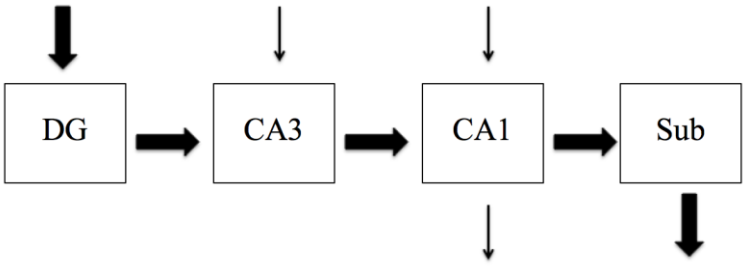

Figure 1. Schematic diagram of major input and output regions of hippocampal formation (HF)

The figure is a schematic diagram of the sequence in which major input and output regions of the hippocampal formation (HF) are activated by input from the entorhinal cortex. Bolded arrows are indicative of major input/output regions, and smaller arrows represent areas that receive minor input/output. Dentate gyrus (DG) serves as the major input area of the HF, and subiculum (Sub) serves as the major output area.

The purpose of this study was to investigate the influence of EE on neural activation in the HF excitatory pathway, evoked by an OPP task. This task was used to assess the effects of EE on exploration, object recognition, and novelty preference, as the rat was able to explore and interact with newly and familiarly placed objects. Based on relevant research, it was thought that enrichment would produce differences in the level of activation observed in major input and output regions of the HF, including DG, CA3, CA1, and Sub, following performance of an OPP task. Because EE provided an opportunity for rats to interact with novel and familiar objects and conspecifics, it was posited that regions of the HF of enriched rats would exhibit less reactivity to novelty provided by the OPP task.

2. Materials and Methods

2.1 Subjects

Adolescent Long-Evans hooded rats (*n*=30, 15 male, 15 female), housed in plastic shoebox cages in a humidity-controlled vivarium (12:12 hr. light-dark cycle), were used as subjects. Food and water were supplied *ad libitum*. Subjects were bred and cared for by the Arts and Sciences Animal Facility at Appalachian State University. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Appalachian State University (#13-06, #15-02).

2.2 Environmental Enrichment

Between postnatal days (PNDs) 25 and 48, a group of enriched rats (*n*=16, 8 male, 8 female) received a total of 18, 90 min EE sessions. EE took place in a 45.7 X 48.3 X 78.7 cm (w X d X h) wooden frame and 1/2-in. hardware cloth cage. Objects of varying size, shape, and color were arranged on the floor and platforms of the cage, as well as hung from the cage ceiling (Fig. 2). Platforms were located at 14.0, 24.8, 43.2, and 61.0 cm above the floor of the cage and were accessible via hardware cloth ramps. The arrangement of objects was varied daily, according to four different set-ups that were alternated throughout the 18 EE sessions. EE sessions were given in groups of 8 same-sex conspecifics from multiple home cages to allow for the interaction with familiar and unfamiliar rats. EE provided opportunities for novelty exposure, as well as physical and social stimulation.

A group of age-matched control rats (*n*=14, 7 male, 7 female) did not receive enrichment. Instead, they were handled twice on each EE day to control for possible confounding effects of handling, since enriched rats were regularly

handled by experimenters during the transfer between cages.

Figure 2. Example enrichment cage with objects of varying size, shape, color, and texture.

The photograph shows an example female enrichment cage with one of the four designated set-ups. Objects featured in this particular set-up include a tennis ball, PVC pipe, foam football, and others. The male enrichment cage (not shown in this figure) was designed as a mirror image of the female enrichment cage, featuring an identical arrangement of objects. Objects such as food and running wheels were excluded from enrichment cages.

2.3 Object Place Preference Task

All subjects underwent an OPP task on pnd 49 prior to being sacrificed. The OPP task was conducted in a 1-m^2 open field marked by an 8 X 8 grid with four distinct objects present (Fig. 3). During the first trial of a two-trial procedure, a rat was placed in the center of the field and allowed to explore the enclosure/objects for 3 min. The rat was then placed back into its home cage for a delay period of 30 min between trials.

Trial 2 of the OPP task was used to evoke activity in the hippocampal formation (HF) prior to sacrifice. In this trial, the positions of two objects used in Trial 1 were exchanged, while the additional two objects remained in their original positions. During Trial 2, the rat was placed back in the center of the field and allowed to explore the enclosure/objects for 3 min. The time in which a rat spent contacting the newly placed, versus familiarly placed, objects was assessed in a separate behavioral study.

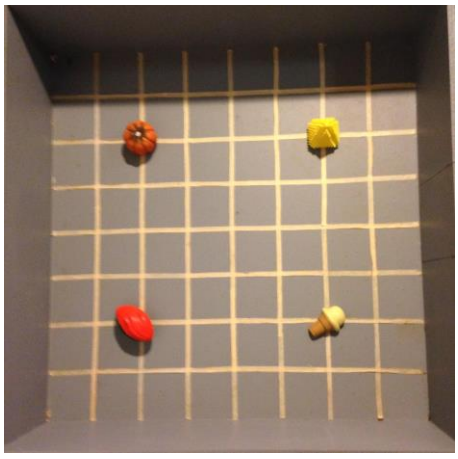

Figure 3. Object place preference (OPP) task apparatus

This figure shows the open field containing four distinct objects in which an object place preference (OPP) task was conducted. In this example, objects in the field included a synthetic pumpkin, ice cream cone, foam football, and plastic pyramid. The positions of the pumpkin and ice cream cone were switched for Trial 2 of the task.

2.4 Histology

Following the OPP task, subjects were placed in quiet and dark conditions for 60 to 90 min, prior to receiving a lethal injection of sodium pentobarbital (100 mg/kg b.w., ip). Upon the absence of tail and corneal reflexes, subjects were intracardially perfused using phosphate buffered saline (PBS), followed by 4% paraformaldehyde in 10 mM phosphate buffer (PB). Brains were extracted from the skulls and stored in 4% paraformaldehyde and 10% sucrose at 4 °C for one week, then transferred to PB solution and stored at 4 °C until tissue was cut.

Sagittal sections (50 µm) were cut from individual hemispheres using a Vibratome® Series 1000 Sectioning System and floated in PBS. Selected sections were later processed using immunohistochemistry (ihc) to visualize the expression of c-Fos protein, a marker of neural activation.

On Day 1 of ihc, sections were rinsed in PBS (2 x 5 min), followed by 0.5% hydrogen peroxide in water for 15 min. After two additional rinses in PBS (2 x 5 min), sections were incubated in 15% goat serum with 0.25% Triton-X for 60 min. Sections were then floated in rat anti-c-Fos made in rabbit (Santa Cruz, SC-52) at 4 °C for approximately 40 h.

On Day 2 of ihc, sections were rinsed in PBS (6 x 10 min) and incubated in biotinylated goat anti-rabbit secondary antibody (Vector Labs) for 60 min, followed by additional rinses in PBS (3 x 10 min). Sections were incubated in peroxide-labeled avidin-biotin complex (ABC, Vector Labs) for 1 h and rinsed again in PBS (2 x 10 min). Lastly, sections were exposed to the VIP enzyme substrate (Vector Labs) for at least 2 min. Sections were then removed from the enzyme and floated in cold PBS until they were mounted onto gel-coated slides. Once mounted, sections were dehydrated in a graded series of ethanol solutions, cleared with toluene, and cover-slipped using Permount (Fisher).

2.5 Microscopy And Data Analysis

Neural densities, defined by the number of c-Fos positive (Fos+) neurons within a series of sampling frames, were quantified using stereological techniques to compare levels of activation between enriched and non-enriched rats. Microscopy was conducted using a Nikon Eclipse microscope and PixeLink digital camera. Images were viewed at a Plan 10 objective and 1024 x 768 pixel image size. Structures of the HF were identified using an atlas of the rat brain[14].

Darker cells generally indicated a greater presence of c-Fos protein. The relative darkness of cells was used to categorize the level of neural activation, such that a larger proportion of dark (fos+) cells within a sampling frame was used to identify areas of greater activation[16]. For the purpose of this study, only the number of dark cells present in a sampling frame was included in the final dataset.

Prior to counting, experimenters identified the darkest cell and the “lightest dark” cell within a sampling frame to use as markers. Individual markers for each section were used to reliably characterize cells as dark, versus light or medium, in appearance.

Cell counts were obtained by marking individual neurons that were present within a series of sampling frames (200 x 200 µm) fixed over top of the computer screen through the depth of a tissue section (50 µm). Cells present on the top and/or right borders of the frame were included in the cell counts, while cells present on the lower and/or left borders of the frame were excluded. The number of dark cells in each sampling frame was recorded and averaged for each section.

The above procedure was repeated for all HF structures of interest, including DG, CA3, CA1, and Sub, to obtain an average density of fos+ neurons for each structure. Usable neural densities from regions of the HF were obtained for 12 enriched and 10 non-enriched rats, and a database was made using Microsoft Excel. For each HF structure, the density of fos+ neurons per section was compared between enriched and control rats using an independent samples t-test.

3. Results

Considering the HF as a whole, a history of EE reduced neural activation by 28.5% in comparison to non-enriched controls as indicated by densities of fos+ neurons (EE M=21.4, SD=17.1 and control M=29.9, SD=14.7). The reduced neural activation within the HF of enriched rats was observed qualitatively (see Fig. 4) and quantitatively (see Fig. 5).

On average, fewer Fos+ neurons were observed within the 200 x 200 x 50µm sampling volumes of DG, CA3, CA1, and Sub from enriched in comparison to control rats.

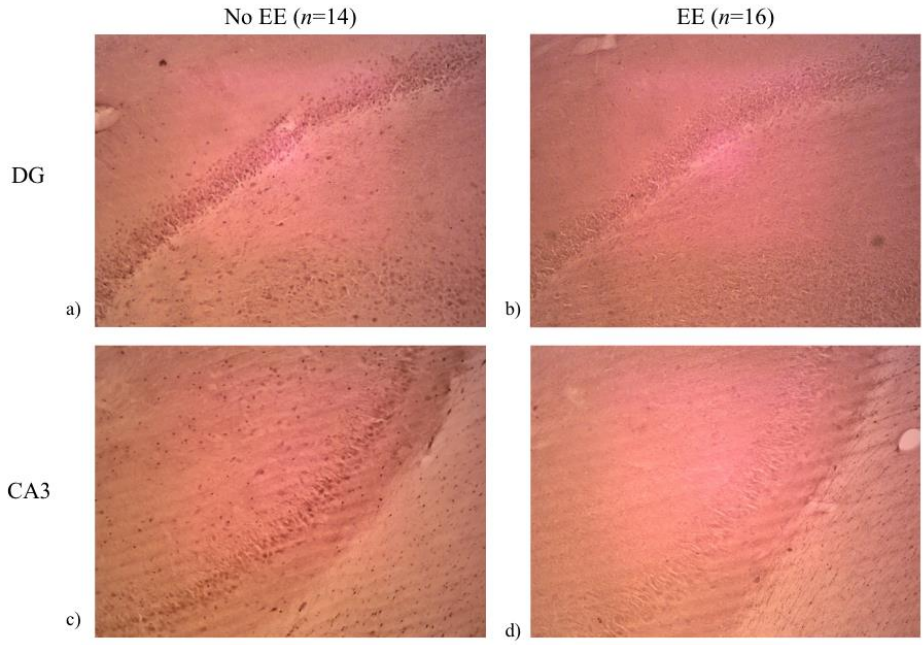

Figure 4. Images of Fos+ neurons in hippocampal formation (HF) of enriched and non-enriched rats

The photomicrographs are example images of Fos+ neurons in dentate gyrus (DG) and CA3 regions of the hippocampal formation (HF), showing relative differences in activation between enriched and non-enriched rats. a) The DG region of HF in the non-enriched rat showed a visibly greater proportion of fos+ neurons, as compared to DG in an enriched rat (shown in 3b). b) DG exhibited relatively few Fos+ neurons in an enriched rat. c) A greater proportion of fos+ neurons were observed in the CA3 region of a non-enriched rat, as compared to an enriched rat (shown in 3d). d) The CA3 region of an enriched rat showed relatively few Fos+ neurons.

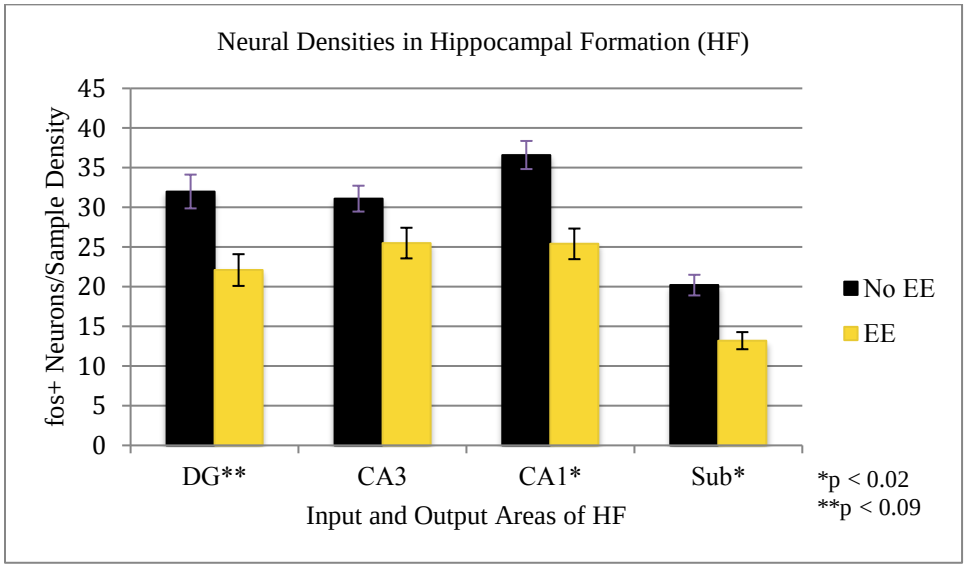

Figure 5. Mean neural densities within hippocampal formation (HF) of enriched and non-enriched rats

Figure 5. Mean densities of fos+ neurons, per 200 x 200 x 50 µm tissue sample, within DG, CA3, CA1, and Sub regions of the hippocampal formation (HF) in enriched and non-enriched rats.

In one primary input area, DG (30.9% reduction, $t(47)=1.73$, $p < 0.09$), and an internal processing area, CA3 (18.0% reduction, $t(52)=1.28$, $p < 0.21$), neural activation change in EE rats from controls was not statistically significant. However, in CA1, a processing and output region (30.6% reduction, $t(57)=2.40$, $p < 0.02$), and Sub, a primary output zone (34.7% reduction, $t(57)=2.45$, $p < 0.02$), a history of EE significantly reduced Fos+ neuron densities evoked by the OPP task when compared to those in control HF. In general, evoked neural activation, as indicated by Fos+ neuron densities, was reduced progressively through the pathway (see Fig.1 and Fig. 5).

4. Discussion

Given the opportunities to explore objects in novel arrangements during EE, it was hypothesized that enriched rats would exhibit less reactivity to novelty, due to the possible habituation of such experience. As a result, the repeated activation of the HF during EE may have allowed rats to utilize this pathway more efficiently over time. These findings are supported by existing literature, which suggests that exposure to EE during adolescence may alter the efficiency of structures in the HF, such that fewer neurons in a given structure must be activated in order to process novel information[22]. In other words, EE was shown to promote adaptation to the rearrangement of objects in an animal's environment. While these findings contrast with prior studies of EE among adult rats, which describe an increase in novelty preference as a result of EE, it is important to note the observed divergence between enrichment effects in adolescents and adults, as described by van Praag[6,20].

The hypotheses that EE would produce significant changes in the HF pathway, and would effectively attenuate activation in the HF structures of interest, were supported. However, these changes were only significant in CA1 and Sub regions. Since DG and CA3 are activated early in the HF pathway (Fig. 1), relative to CA1 and Sub[4,21], it is possible that the sequence of activation in the HF may explain the less robust changes observed in these two structures. Additionally, prior research suggests that neural activation may be altered differently between DG and CA1 structures, in response to novel environments[13]. Nitz & McNaughton, for example, found that neural activation was increased in DG and was suppressed in CA1 following enrichment[13].

Given the consideration of DG as a major input area of the HF, as well as its role in identifying novel spatial relationships, the lack of significant differences in the activation of this structure may indicate that EE and non-EE groups were equally able to recognize novel aspects of the environment[4]. This explanation may also be true for CA3, as it is activated relatively early in the HF pathway and plays a role in novelty detection, similar to DG[21]. According to VanElzakker et al., the lack of significant differences in the activation of DG and CA3 structures, between enriched and non-enriched groups, may be explained by the structures' role in identifying novel features of the environment, which did not likely differ between groups[21].

Given the role of CA1 and Sub in processing novel spatial information, the significant differences in activational changes within these structures may be interpreted by a divergence in the amount of information processed between the EE and no-EE groups[9,21]. Since enriched rats received opportunities to process novel aspects of the environment during EE sessions, it is possible that with consistent practice, enriched rats were able to process information more efficiently, requiring less activated neurons in CA1 and Sub structures to accomplish certain behavioral or cognitive outcomes[22]. Additionally, since CA1 and Sub receive input from at least two structures in the HF pathway, their relative positions in the sequence of activation may account for the more robust differences observed in these two structures[22].

The results of the current study suggest that a history of EE may effectively suppress activation as a neural signal moves sequentially throughout the HF pathway, such that significant differences relative to not enriched brains are observed in CA1 and Sub, which are activated later in the pathway. Over time, periodic enrichment during adolescence may result in more efficient usage of circuits associated with spatial memory, in addition to improvements in spatial learning and memory and object recognition[4,7,8,20]. These findings support the existing literature of EE regarding adolescent rats, which describes suppression in novelty preference and highlights the relevance of adolescence as a critical period for the study of enrichment effects[3,4]. Thus, a history of EE may provide significant implications on novelty preference and risk-taking behaviors, as well as spatial learning and memory, during adolescence.

5. Acknowledgements

The authors wish to express their appreciation to the Department of Psychology at Appalachian State University for providing the necessary resources to conduct this research as well as past members of the Zrull Lab, particularly Dana Cobb, for work that generated the tissue used in this research. They also express their appreciation to faculty advisor Dr. Mark C. Zrull for his guidance, feedback, and support throughout this project.

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