Effects of Environmental Enrichment on Rat Behavior in the Open Field Test

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Abstract

The open field test (OFT) is used to measure exploratory behavior and activity in rodents. Behaviors such as thigmotaxis (staying close to the walls of the field) and defecation are cues of anxiety or fear while time spent in the center of the field and high locomotor activity indicate exploratory behavior. Environmental enrichment (EE) is often used to attenuate behavioral deficits after brain injury; however, less is known about behavior changes caused by EE in normal animals. Conducting an OFT on normal and enriched adolescent rats may provide insight into the effects EE has on exploration of a novel environment. Male (n=4) and female (n=4) Long-Evans rats were exposed to EE with familiar and unfamiliar same-sex conspecifics for 1.5 hours on a two days on one day off schedule for 30 days starting on post-natal day (pnd) 34. The enrichment scheme consisted of variable placements of toys (e.g., shoestring, tennis ball, hanging objects) in an enclosure with four levels connected by ramps. On pnd 65, rats were placed into a forced-entry open field, which consisted of a dimly lit 1-m$^2$, 8x8 grid of squares. Non-enriched males (n=4) and females (n=4) were also tested. To control for anxiety, data was collected from the last 3 minutes of the 5 minute OFT. OFT measurements included: time spent in outer squares, time spent in inner squares, rate of movement, time spent grooming, and number of rears and wall climbs. Enriched rats spent 56.6% more time in inner squares than non-enriched rats (p<0.05) and spent 18.7% less time in the outer squares than non-enriched rats (p<0.05). Additionally, enriched rats entered 137% more inner squares than non-enriched rats (p<0.05). These results indicate increased exploratory behavior in adolescent, enriched rats in a novel environment. It is likely that repeated EE through exposure to an often changing environment aided in inhibition of initial fear response when introduced to a new novel environment. EE has been shown to activate structures of the medial prefrontal cortex, which is involved in fear response inhibition.

Keywords: Enrichment, Behavior, Environment

1. Introduction

This study was conducted to gain insight into the effects of environmental enrichment during adolescence on exploratory behavior in rats. Environmental enrichment (EE) has been shown to alter various aspects of behavior in injured and non-injured animals. In research with injured animals, EE prior to injury provided protection against behavioral deficits that resulted from brain lesions$^6$, and EE after injury promoted recovery of learning capacity that was lost due to brain lesions$^{13}$. EE impacts the exploratory behavior of both normal adult and adolescent rats$^8,9,10,12$ and is usually assessed with the open field test (OFT), which is a standard test that is used to observe exploratory behavior and general activity in rodents$^4$. Adult animals reared in an enriched environment generally display a decline in locomotor activity$^{7,9}$ and altered movement patterns$^{12}$ in the OFT as compared to animals reared in a barren environment. While experience with novel environments influences behavior in adult and adolescent rats$^{5,10}$, it is worth noting that non-enriched adolescent rats exhibit more locomotor and exploratory activity than their non-enriched adult counterparts$^{10}$, which indicates some general differences between adolescent and adult animals in
exploratory behavior. Learning and spatial memory are also affected by EE, as shown by a decrease in escape latencies in the Morris Water Maze task\textsuperscript{7,9} in normal rats with enrichment experience in comparison to non-enriched controls.

It is widely acknowledged that changes in behavior are a result of underlying anatomical or chemical changes in the brain. For example, changes in learning and memory in rats reared in an enriched environment have been linked to increased neurogenesis and changes in neurotransmitter levels in the hippocampus\textsuperscript{9,11}. EE also increases activation of certain brain structures, which may lead to behavioral changes. When a single session of EE was administered to adult transgenic mice, activation of the medial prefrontal cortex (mPFC) was increased, which is associated with inhibiting fear response\textsuperscript{1,3}. The inhibition of a subject's fear response due to activation of mPFC can lead to a congruous alteration of behavior, such as decreasing freezing and enhancing learning under stress\textsuperscript{2}. Similarly, researchers were able to show that EE actually enhanced seizure activity linked to activation of the amygdala but decreased fear behavior\textsuperscript{14}, which is dependent on amygdala function and thus impacts exploratory behavior.

There is a wealth of research about the effects of EE on animal subjects' behavior and the neural correlates of that behavior. It is most common to find studies using adult subjects, injured subjects, and animals that are raised in enriched environments and then tested as adults. While some studies have focused on younger brain injured subjects\textsuperscript{14} or normal young animals living in enriched environments\textsuperscript{10}, less is known about the behavioral effects of EE on non-injured, adolescent subjects who experience EE on only a part-time basis. In this study, an OFT was administered to animals just prior to adulthood in order to assess differences in behavior in normal rats experiencing a series of EE sessions as adolescents as compared to normal non-enriched rats of the same age with the hope of better understanding the impact of enrichment on unlearned, exploratory behavior in this less-studied age group. The degree of exploration and comfortability was determined using previously established measures such as time spent away from the periphery of the field, number of rears/wall climbs, time spent grooming, and rate of movement\textsuperscript{4}.

Based on previous research, it was hypothesized that EE rats would exhibit more exploratory behavior and comfortability than non-EE rats. Specifically, we expected that EE rats would spend more time away from the periphery of the field (i.e. more time in inner squares and less time in outer squares, see Method for description of field), exhibit a higher number of rears and wall climbs, and spend more time grooming than non-enriched rats. However, it was hypothesized that the rate of movement for enriched rats would decrease in comparison to non-EE rats (i.e. less area of the open field would be traversed by EE rats), as that has been the pattern observed in the literature\textsuperscript{7,9,12}, despite the decrease in the fear response of freezing seen in enriched rats\textsuperscript{14}.

2. Materials and Methods

2.1 Subjects

Sixteen Long-Evans hooded rats, eight male and eight female, were housed in plastic shoebox cages in a temperature-controlled vivarium with a 12 hour on/12 hour off light/dark cycle. Food and water were unrestricted. The rats were randomly assigned to one of the two EE conditions, which were balanced by sex. After the enrichment phase of the study, all rats were administered the OFT.

2.2 Environmental Enrichment

Beginning on postnatal day (pnd) 34, 8 rats (4 male, 4 female) were placed in one of two identical cages with familiar and unfamiliar same-sex conspecifics. The rats engaged in EE for 1.5 hours a day in a two days on, one day off schedule for 30 days, resulting in a total of 21 EE sessions. The enrichment scheme consisted of variable placements of toys (e.g., shoestring, tennis ball, and hanging objects) in an enclosure with four levels produced with platforms connected by ramps (Figure 1). Placement of toys was changed regularly according to a prewritten schedule. Non-enriched rats were handled for approximately 5 minutes on EE days to control for handling needed to move EE rats between home cages and the enrichment enclosures.
Figure 1. Pictures of cages in which rats were enriched: a) outside of EE cages; b) inside of EE cages. Each cage has four levels connected by ramps and contains objects with which the rats can play. The objects were moved regularly according to a prewritten schedule to avoid habituation.

2.3 Open Field Test

The OFT was administered in a dimly lit 1-m$^2$ arena, which was sectioned into an 8x8 taped grid to aid in measuring movement (Figure 2). On pnd 65, each subject was placed individually into the center of the open field to begin a 5 minute trial. Each trial was videotaped for later data analysis, and behaviors indicative of exploration were measured for each OFT session. These behaviors included number of inner and outer squares entered, time spent in the outer versus inner squares of the field, number of rears and wall climbs, and the time spent grooming. Each OFT trial was split into two parts, the initial 2 minutes and the last 3 minutes. To allow initial habituation to the field, measurements from the last 3 minutes of each trial were used to assess behavioral differences between enriched and non-enriched rats.
2.4 Data Analysis

Videotapes of OFT sessions were viewed multiple times by two raters to record values for various measures of exploratory behavior for each rat. Event frequencies were tallied with the aid of mechanical counters, and event durations were recorded using digital stopwatches. The variables measured included the number of inner and outer squares entered, the time spent in inner and outer squares of the field, the number of rears, the number of wall climbs, and the time spent grooming. Hypotheses about differences between the behavior of EE and non-EE groups were assessed using an independent samples t-tests.

3. Results

The hypothesis that enriched subjects would spend more time away from the periphery of the open field was supported by the data. Enriched rats spent significantly less time along the periphery of the field ($t_{14}=2.77, p<.05$) and thus spent significantly more time in the inner squares ($t_{14}=2.76, p<.05$) than their non-enriched counterparts (see Figure 3). While it was hypothesized that the number of rears and wall climbs would increase in enriched animals as an indication of increased exploration, no significant difference between EE and not enriched rats was found for either variable. The number of rears ($M=3.9, SD=3.1$ for both groups) and wall climbing episodes ($M=11.9, SD=4.0$ and $M=10.8, SD=4.5$ for EE and non-EE, respectively) were similar. EE rats did spend more time grooming ($M=30.8$ seconds, $SD=19.2$ seconds) than non-EE rats ($M=19.3$ seconds, $SD=16.2$ seconds); however, the difference between the groups was not statistically significant, and the prediction that EE rats would spend more time grooming than non-enriched rats was not supported.
Figure 3. The data gathered shows that EE rats spent significantly more time in the inner squares as compared to non-EE rats ($t_{14}=2.77, p<.05$). Accordingly, EE rats also spent less time in the outer squares than their non-enriched conspecifics ($t_{14}=2.76, p<.05$).

The rate of movement was expected to decline in EE rats, however the data showed the opposite trend. While a significant difference was not found between groups in the number of outer squares entered ($t_{14}=1.26, p>.05$), there was a significant difference in the number of inner squares entered ($t_{14}=2.77, p<.05$). EE rats moved through more than twice as many inner squares as did non-EE subjects (see Figure 4).

Figure 4. The data gathered shows that EE rats spent significantly more time in the inner squares as compared to non-EE rats ($t_{14}=2.77, p<.05$). Accordingly, EE rats also spent less time in the outer squares than their non-enriched conspecifics ($t_{14}=2.76, p<.05$).

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Figure 4. This figure shows the number of squares entered during open field trials. A significant difference was not seen between groups in the number of outer squares entered ($t_{14}=1.26, p>.05$), but EE rats moved through more than twice as many inner squares than non-EE rats ($t_{14}=2.77, p<.05$).
4. Discussion

The overall hypothesis that exploratory behavior would increase in enriched subjects had some support from the data, but not all of the hypotheses concerning specific behavioral differences were supported. EE rats did spend significantly more time away from the periphery of the open field, but there was no significant difference in number of rears or wall climbs, or in time spent grooming. Furthermore, the hypothesis that rate of movement would decrease, based on previous research, was not supported, but this result adds to the increased exploration hypothesis.

The expectation that EE rats would spend significantly more time in the inner squares and, consequently, less time in the outer squares than non-EE rats, was supported by the data. Behaviorally, increased exploration of the interior of the open field by EE rats in comparison to non-EE animals likely reflects their level of experience with novel environments. This could also be due to a decrease in fear response, which may be caused by increased activation of the mPFC.

While the data supports the expectation of EE rats spending more time away from the periphery of the field, the hypotheses regarding the number of rears and wall climbs and the time spent grooming were not supported. In addition to time spent away from the edge of the open field, wall climbs are considered a sign of exploratory behavior, yet there was not a notable difference in expression of this behavior. This could be a by-product of the decreased time spent near the walls of the open field. Time spent grooming in the OFT, another behavior unaffected by EE in this study, is seen as a sign of comfort within the environment. An explanation for this may be that EE inhibits the fear response associated with a novel environment, but does not increase the level of comfort of the subject (i.e. there is still some anxiety, but fear of exploration is reduced). Another explanation may simply be that adolescent rats are more active in the OFT as compared to their adult counterparts, and because grooming cannot happen while a rat is moving it is not as affected by EE of adolescent rats.

The hypothesis that locomotor behavior would decrease in EE rats was rejected by our data, however increased locomotion fits in with the overall hypothesis of increased exploratory behavior in EE rats. There are multiple possible explanations for the observed increase in locomotor behavior. One such explanation is that the increased rate of movement is merely a by-product of the decreased freezing response due to activation of the mPFC. This would account for the difference between groups, but not for the difference between this study and the results from previous studies. One source of discrepancy could be that in other studies, EE rats were continuously housed in the EE cages for the entire duration of the enrichment procedure, whereas the rats in this study were housed in separate shoebox cages and only placed in EE cages for 1.5 hours on EE days. If rats are continuously housed in an enriched environment, it ceases to be novel, therefore decreasing their relative experience with novel environments. This relative inexperience with novel environments would lead to a decrease in exploration. Lastly, the increased rate of movement could be due to the rats being adolescents as opposed to adults, as adolescents are generally more active and exhibit more exploratory behavior than adult rats.

While all of the hypotheses regarding specific behavioral differences were not supported, the data seemed to support the overall hypothesis of increased exploration in EE rats. An interesting extension to this study would be to examine the extent to which EE stimulates brain structures, specifically those related to fear response and novelty seeking, over time. Also of interest would be how these changes differ across stages of development (i.e. would EE affect juveniles, adolescents, adults differently over time?). As this is just a preliminary study regarding the effects of EE on general exploratory behavior in normal adolescent rats, it is difficult to determine a long-term application; however, with the current findings and with further research regarding more specific behavioral and neurological effects of EE, the future of EE looks exceedingly bright.

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6. References