

Spring 2009

Enriched Environments Protect Against Depression Brought About By Chronic Mild Stress and Increase Neuronal Density in the Hippocampus in Sprague-Dawley Rats

McKenzie LeTendre
Regis University

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ENRICHED ENVIRONMENTS PROTECT AGAINST DEPRESSION BROUGHT ABOUT BY
CHRONIC MILD STRESS AND INCREASE NEURONAL DENSITY IN THE HIPPOCAMPUS IN
SPRAGUE-DAWLEY RATS.

A thesis submitted to the Regis College Honors Program in partial fulfillment of the
requirements for Graduation With Honors

By

McKenzie LeTendre

May 2009

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Acknowledgements

I would like to thank Dr. Mark Basham, my advisor, Dr. Jose LaFosse, my reader and Dr. Tom Bowie, the director of the Honors Program for their guidance and support in the completion of this project.

I would like to thank the SPARC committee for funding for this project and Regis University for the use of their facilities during this project.

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Abstract

Enriched environments (EE) integrate complex housing conditions with social stimulation and are shown to ameliorate symptoms of depression in rats with as much success as pharmacological treatment. Furthermore, rearing in EE is associated with an increase in hippocampal neurogenesis. This study attempted to demonstrate the depression related behavioral effects of rearing in EE in comparison to rearing in social isolation (SI) using a sucrose preference test (SPT) and forced swim test (FST). I hypothesized that rats reared in EE would exhibit less anhedonia and behavioral despair during and after exposure to a chronic mild stress (CMS) procedure and would show an increased density of neurons in the hippocampus. The rats were reared for 61 days in either EE or SI conditions and then exposed to CMS for 14 days. Behavioral measures were taken during and after CMS. Upon completion of the behavioral study, three rats from each condition were sacrificed and neuronal density in the hippocampus was determined. I found that EE prevented behavioral despair demonstrated by the FST and that EE increased the density of neurons in the hippocampus providing a possible mechanism for the behavioral effects of EE.

Introduction

Major depressive disorder affects 2%-5% of the United States population, while up to 20% percent of individuals living in the United States suffer from more mild forms of depression (Nestler, et al., 2002). Suicide is the cause of death in approximately 15% of people with some form of major depressive disorder, making this disorder one of serious consequence (Manji, Drevets, & Charney, 2001). Understanding the neural pathology implicated in depression in addition to the external factors involved in the disorder are helpful in understanding the behavioral syndrome, as well as learning how it can be treated and even prevented. Depression is caused by the interaction of genetic susceptibility and environmental factors; however, current explanations of the disorder are purely descriptive. In the past, this interaction between environment and genetic predisposition was considered on a neurochemical basis, but recent research suggests a connection between regional reductions in the volume as well as size of neurons in discrete central nervous system structures (Sapolsky, 2001). Furthermore, depression is associated with impairments in structural plasticity and cellular resilience indicating structural deficits in the central nervous system are implicated in depression (Manji, et al., 2001).

The cellular basis of environmentally regulated structural remodeling of the hippocampus provides a new window into processes that are undoubtedly involved in the etiology and treatment of depression. One environmental factor of particular interest to the development of depression is housing conditions. Early life aversive experiences are a

significant risk factor in the development of depression in both humans and animals (Sanez, Villagra & Trias, 2006). The most prominent postnatal manipulation associated with the development of abnormal or depression-related behavior is the housing condition in which animals are reared (Sanez et al., 2006). Enriched environments have a positive effect on both behavioral and physiological measures of depression (Chourbaji, Zacher, Sanchis-Segura, Spanagel, & Gass, 2005). Since early life aversive experience in the form of social isolation produces a risk for depression (Sanez, et al., 2006), it is possible that early life positive experience in the form of enriched housing conditions produces a protective buffer against depression.

Severe stress is also associated with an increased risk for depression. Thus, one of the most common ways to produce a depressive syndrome in rodents and primates is to present a series of stressors (Nestler et al., 2002). This procedure, often referred to as the chronic mild stress (CMS) paradigm administers a series of unpredictable stressors, intended to mimic daily stressors in humans that are reported to contribute to the onset of depression. The CMS procedure reliably produces physiological and behavioral changes paralleling some of the symptoms of depression as listed in the DSM-IV (Willner, 1997). Depression-related symptoms associated with the CMS procedure are: a decrease in aggressive and sexual behavior (D'Aquila, Brian, & Willner, 1994), an overactive responsiveness of the immune system (Siberman, Ayelli-Edgar, Zorrilla-Zubilete, Zieher & Genaro, 2004), and increased secretion of corticosterone (Ayensu, et al., 1995), disruptions in REM sleep episodes and reduced REM sleep latency (Gronli, Bramham & Murison, 2006) and increased activity in the hypothalamic-pituitary-

adrenal (HPA) axis (Muscat & Willner, 1992). All of these symptoms are reversed by treatment with antidepressants (Willner, 1997), suggesting that the CMS procedure is an effective model for depression in rodents.

Within the CMS model of depression, reliable ways to measure depressed behavior have been developed. More than 30 years ago, McKinney and Bunney (1969) established four minimum requirements for animal models of depression that have been widely cited. The four criteria state that depression in an animal is (1) reasonably analogous to the symptomatology in humans; (2) there is a change in behavior that can objectively be monitored (3) the changes in behavior can be reversed by the same modes of treatment that are effective in humans; and (4) the behavioral syndrome is reproducible between laboratories. In line with these criteria, this research will focus on two major symptoms of depression as cited in the DSM–IV: markedly diminished interest or pleasure in all or most activities and depressed mood (American Psychiatric Association, 1994).

Anhedonia, the loss of interest in behavior that is normally stimulating, is consistently demonstrated in depressed rats. The most extensively used behavioral measure of anhedonia is a sucrose preference test (SPT), which measures the rat's affinity for a sweet sucrose solution. This solution is normally rewarding to rats, and a decreased consumption is interpreted as a decrease in hedonic behavior, or anhedonia (Pucilowski, Overstreet, Rezvani, & Janowsky, 1993). Anhedonia evident through the sucrose preference test in depressed rats is reversed by

treatment with antidepressants, and has been frequently used in understanding depression in rodents (Slattery, Markou & Cryan, 2007).

Behavioral despair is analogous to 'depressed mood', in rats and is most readily expressed in response to a stressful stimulus (Lucki, 1997). A forced swim test (FST) is the most commonly used behavioral measure to test for behavioral despair. In this test, rats are placed in an inescapable cylinder of water and will usually make initial attempts to escape, but then develop an immobile posture. Immobility reflects a lack of escape directed behavior indicating behavioral despair (Cryan, Markou & Lucki, 2002) The FST is very effective at predicting the antidepressant efficacy of new medications (Nestler, et al., 2002) and treatment with antidepressants causes the rat to actively persist in escape directed behaviors, validating the model as an effective measure of behavioral despair (Porsolt, 2000).

The hippocampus is implicated in the biological pathology of depression, and is extremely susceptible to environmental factors because it is one of the most plastic structures of the brain and is particularly vulnerable to stress and stress hormones (Gould & Tanapat, 1997). Recent research suggests that new neurons are produced in the dentate gyrus of the hippocampus in a variety of mammalian species and that these neurons develop morphological characteristics of hippocampal granule cells, expressing markers of mature neurons (Gould & Tanapat, 1997; Hastings & Gould, 1999). This extended period of cell genesis in the hippocampus makes this structure unusually sensitive to structural changes dependent upon

experience, and give this structure the propensity to be altered by postnatal experience, enabling a certain amount of adaptive plasticity (Gould & Tanapat, 1999).

Adult neurogenesis in the dentate gyrus is particularly sensitive to stress. The major physiological hallmarks of stress: glucocorticoid secretion and NMDA receptor activation, naturally inhibit neurogenesis (Moghaddam, Boliano, Stein-Behrens & Sapolsky, 1994). Acute stress suppresses the neurogenesis of granule neurons in the dentate gyrus and hippocampus proper, and repeated stress causes atrophy of dendrites in the hippocampal complex (McEwen & Magarinos, 2001). In humans, imaging studies report a decreased volume of hippocampal tissue in depressed patients (Bremner, et al., 2000) Furthermore, clinical studies have consistently demonstrated that recurrent depressive episodes, brought about by stress, lead to a reduced hippocampal volume, plausibly due to decreased production of neurons and dendritic atrophy (Lemarie, Koehl, Moal & Abrous, 2000).

Investigating the process of decreased neurogenesis in the hippocampus of rats provides a useful model for the investigation of possible cellular mechanisms underlying the pathology of depression. Although the hippocampus is commonly associated with processes such as learning and memory, recent research suggests that dysfunction of the hippocampus contributes to symptoms of depression and anxiety (Duman, 2004). In humans, neocortex and hippocampus mediate the cognitive aspects of depression, such as memory impairments and feelings of worthlessness, hopelessness, guilt, doom and suicidality (Nestler, et al., 2002). The action of the hypothalamic-pituitary-adrenal (HPA) axis also provides a link between adult

neurogenesis and depression. In stressful situations the HPA axis is one of the primary physiological actions preparing mammals for a behavioral and physical response. More than 50% of depressed patients exhibit an abnormal regulation of this system, resulting in a sustained elevation of cortisol levels (Duman, 1994). Since stress is shown to inhibit neurogenesis through the action of glucocorticoids, and the hippocampus provides negative feedback regulation of the HPA axis, atrophy of the hippocampus in depressed patients could lead to a damaging cycle of HPA hyperactivity (Sapolski, 2001). Furthermore, from a systems model of the brain, the hippocampus is intricately linked with the prefrontal cortex, cingulate cortex and the amygdala, all of which contribute to alterations in mood and emotion (Manji, et al., 2001). Clearly, there are a number of mechanisms providing a link between depression related behavior and structural changes in the hippocampus.

Contrasting the effects of chronic stress, enriched environments produce the opposite effects on hippocampal structure and function. Enriched care environments combine social stimulation with complex inanimate objects for animals to interact with (van Praag, Kempermann, & Gage, 2001). While depression produced by the CMS paradigm is conclusively linked to a decrease in hippocampal neurogenesis, enriched environments promote an increase in hippocampal neurogenesis, significantly altering physiological parameters relevant for stress-related disorders such as depression (Chourbaji, et al., 2005). Rats housed in enriched conditions produce neurons in the dentate gyrus at significantly higher rates than rats housed in standard conditions and demonstrate a reduction in spontaneous apoptotic cell death in the hippocampus. Rats exposed to prenatal stress show impaired hippocampal long-term

potentiation, but when they are treated with enriched environment, they demonstrate a recovery to control levels (Yang, et al., 2006). Treatment with enriched environments also reverses the effect of prenatal stress on HPA axis reactivity in rats (Morley-Fletcher, et al., 2003). Findings such as these have led researchers to believe that enhanced hippocampal neurogenesis is one of the major mechanisms mediating the antidepressive behavioral effect of enrichment (Jayatissa, Bisgaard, & Tingström. 2006; Hattori, et al., 2007).

The morphological changes in the hippocampus brought about by enriched environments produce an antidepressive and anxiolytic like behavioral effect in rodent models of depression (Segovia, Yague, Garcia-Verdugo & Mora, 2006; Sanz, et al., 2006). Mice housed in enriched conditions showed a reduced immobility time on a forced swim test demonstrating an increased tolerance against an inescapable, stressful situation (Hattori et al., 2007). Enrichment also reduces abnormal behavior such as emotional reactivity and behavioral despair in rats (Chapillon, Patin, Roy, Vincent, & Caston, 2002). Furthermore, enriched environment treatment in the early postnatal stage counteracts cognitive deficits brought about by early life stress in animals (Laviola, et al., 2004).

Enriched environments have successfully been implemented as a treatment for depression, but never as a preventative measure. Considering that the behavioral development of any animal is influenced by environmental factors, social contact, and other external elements (Varty, Paulus, Braff, & Geyer, 2000), and that early life aversive experiences are a risk factor in the development of depression in both humans and rodents, (Wong & Licinio, 2001) it

is likely that early life constructive experience might contribute to the development of a protective effect against depression. Moreover, considering the structural changes in the hippocampus that underlie depression it is probable that the enhanced neurogenesis of the dentate gyrus, and survival of these newborn cells might act as a defensive mechanism against the decreased neurogenesis apparent in depression. Furthermore, the hippocampus shows a considerable amount of plasticity in adulthood (Gould & Tanapat, 1999) making this structure of particular interest in the investigation of the development of a protective factor against depression in adolescent rats. In light of this research, this study hypothesizes that rats reared in enriched environments will demonstrate an increase in hippocampal neurogenesis which will act as a buffer against the decreased neurogenesis present in depression, and that rats reared in enrichment will be more resilient to the depressive effect of the CMS procedure as demonstrated by decreased immobility time in the FST and increased sucrose consumption in SPT.

Method

Animals

Twenty-five 27 – 31 day old male Sprague-Dawley rats (Harlan) were used and maintained under a 12:12 hour light dark cycle (lights turned on at 6:00AM and off at 6:00PM) with free access to food and water unless otherwise specified in the CMS procedure. During days 1-61 of the experiment 13 rats were housed in an enriched environment and 12 rats were housed in a socially isolated environment. Due to an animal housing facility error, rats in the enriched condition were maintained with total illumination from days 1-21. However, this is not expected to negatively impact the study for reasons that will be addressed in the discussion.

Design

Rats were randomly assigned to either socially isolated (SI) housing conditions, n=12, or enriched environment (EE) conditions n=13, and maintained in these conditions for 61 days. At the end of this period, 6 rats from the SI conditions and 6 rats from the EE conditions were exposed to a CMS protocol and housed individually for 14 days. The sucrose preference test and forced swim test were performed during the light phase of the light-dark cycle between 6:00AM and 6:00PM. The first set of behavioral tests were performed at day 7 of the CMS procedure, and the second set were performed the day after the CMS procedure ended at day 15. All procedures were approved by the Institutional Committee for Animal Care and Use of Regis University.

Housing Conditions

SI rats were housed individually in standard polycarbonate cages (8 in height x 9 in width x 18 in length). EE rats were housed in groups of 4 or 5 in a wire cage with a stainless steel bottom (24 in height x 13 in width x 17 in length). The EE cages had three levels made of wire mesh (5 in width x 17 in length) with wire mesh ramps (3 in width x 10 in length) connecting each level. Each EE cage contained 4 non-chewable plastic toys, a running wheel (3 in width x 8 in diameter), one hanging toy with bells attached to the bottom, and one hanging toy with mirrors on all four sides. Every 4 days, the plastic and hanging toys were moved around to create a novel environment.

Sucrose Preference Test (SPT)

During the SPT all animals were housed individually for the 6 hr duration of the test with free access to food. Each cage had one bottle with 30 ml of tap water and one bottle with 50 ml of a 10% sucrose solution on opposite sides of the cage. The placement of the bottles was reversed each time the test was conducted to avoid preservation effects. Sucrose preference was measured by dividing the sucrose solution consumption by the total liquid consumption. After the 6 hr period rats were returned to their home environments.

Forced Swimming Test (FST)

The FST was conducted in a plastic cylinder from which escape was impossible. The apparatus was 32in high, 22in diameter and was filled with 18in of water at room temperature.

Each rat was individually placed in the center of the cylinder and their behavior was recorded for a period of 5 min. During the test, the time spent swimming and the time spent immobile were measured. Immobility was defined as a lack of motion of the body except for small movements necessary to keep the rat's head above water (Sanez et al., 2006).

Chronic Mild Stress Procedure (CMS)

This procedure was adapted from Baker et al. (2006). Stressors were administered for a period of 2 weeks and were applied both day and night. Each day of the week (excluding weekends) animals were confined in a small Plexiglas container for a period of 90 min (3 in height x 3 in wide x 5 in length). During the confinement period a loud noise from a kitchen timer was sounded every 10 min and lasted approximately 5 sec. The confinement occurred at various times each between 10:00AM to 4:30PM in order to maintain the confinement as an unpredictable stressor. Each Wednesday animals were exposed to an empty water bottle for a period of 1 hr following 14 hrs of overnight water deprivation. Water was removed at 7:00 PM each Tuesday night and an empty water bottle was placed in the cage at 9:00AM the following day.

Overnight stressors occurred throughout the week as well. Each Monday night rats were placed in a cage with warm, wet bedding (which cooled to room temperature overnight) beginning at 6:00PM. Standard bedding was replaced at 10:00AM the following day. Each Wednesday and Sunday night rat's home cages were tilted at a 30° angle beginning at 6:00PM and ending at 9:00AM. On weekends (Friday and Saturday) light-dark cycles were reversed so

the room was illuminated from 6:00PM to 6:00AM, and on Sundays the rats were exposed to total illumination for a 24hr period. Normal light-dark cycles were resumed during weekdays.

Table 1. Schedule of Chronic Mild Stress procedure

This table demonstrates the order and approximate time of day that each stressor was administered during the two weeks of exposure to stress.

Time of Day	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
9:00 AM			Empty bottle exposure 1 h		Reversed light dark cycle	Reversed light dark cycle	Total Illumination 24 hrs
11:00 AM	Confinement w/ loud noise every 10 min.						
1:00 PM		Confinement w/ loud noise every 10 min.		Confinement w/ loud noise every 10 min.			
3:00 PM			Confinement w/ loud noise every 10 min.				
5:00 PM			Overnight cage tilt				Overnight cage tilt
7:00 PM	Overnight pairing in wet bedding	Water bottle removed					

Histology

Upon completion of the behavioral studies, 3 rats from the EE/non-stress condition and 3 rats from the SI/stress condition were euthanized with a lethal overdose of sodium pentobarbital (108mg/kg) through an intraperitoneal injection. Animals were then transcardially perfused, first with 0.9% saline and then a 4% paraformaldehyde solution. The brains were removed and placed in 4% paraformaldehyde solution at least overnight. Over a period of one week the brains were soaked in increasing concentrations of sucrose solutions

(10%, 20%, 30%; all in 4% paraformaldehyde). Once the brains sank in a 30% sucrose, 4% paraformaldehyde solution, the brains were embedded in optimal cutting temperature compound and chilled to -20°C . Using a cryostat chilled to -20°C the brains were sliced into $40\mu\text{m}$ coronal sections, and mounted on gelatin coated slides. After warming to room temperature, the slides were rehydrated in a series of ethanol solutions of decreasing concentration, and then soaked in deionized water. Once rehydrated, the slides were placed in a thionin stain consisting of 0.05g thionin/100ml deionized water for 1 min, and were then placed into a 70% ethanol solution containing acetic acid. Next, the slides were rinsed in deionized water and then rehydrated in a series of ethanol solutions. The slides were then soaked in xylene and cover-slipped with preservaslide.

Microscopy and Cell Counting

Neurons in the dentate gyrus of the hippocampus were counted from rats in the SI/stressed condition and the EE/non-stressed condition, because these two groups were expected to have the largest difference in neuronal density. Neuronal counts were made using a Leica DM2500 at 40x magnification. The criterion used to distinguish neurons from glial cells was that a neuron was counted when there were visible processes protruding from the cell body. Sections of tissue from 3 areas per rat were counted: the anterior portion of the dentate gyrus with a sample from between 2.04mm and 2.16mm posterior to bregma, 2mm lateral from the midline and 3.6 mm ventral to bregma, the middle portion, between 3.36mm and 3.48mm posterior to bregma, 3 mm lateral and 3.5 mm ventral to bregma, and the posterior

section, between 4.80 mm and 4.92 mm posterior to bregma, 3.5 mm lateral to the midline and 4mm ventral to bregma. One visual field with an area of 0.238mm² was counted from each section so that there were 3 counts for each rat.

Statistical analysis

Sucrose preference from SPT and time spent immobile from the FST were analyzed using a repeated measures mixed ANOVA design with four levels of the independent condition: SI/stressed, SI/non-stressed, EE/stressed, and EE/non-stressed. The repeated measures were the data from the FST and SPT with 2 levels in each repeated measure consisting of data from behavioral testing time 1 and behavioral testing time 2. Data from the SPT were also analyzed using a t-test to compare all stressed rats with all non-stressed rats. Neuronal counts were analyzed using a t-test to compare total cell counts, and a one-way ANOVA to analyze cell counts by region. All data were analyzed using the SPSS software package.

Results

Forced Swim Test

The data used in this section were the number of seconds each rat spent immobile in a 5 min period. The data recorded from the each of the two sessions of behavioral testing were used.

Table 2. Average number of seconds spent immobile in the FST

Condition	Average seconds immobile (standard deviation)
Testing during week 1	
SI/stressed	124.84 (6.88)
EE/stressed	74.83 (18.77)
SI/non-stressed	66.50 (28.85)
EE/non-stressed	64.00 (31.41)
Testing during week 2	
SI/stressed	135.17 (25.96)
EE/stressed	104.67 (13.95)
SI/non-stressed	83.50 (35.72)
EE/non-stressed	64.71 (26.34)

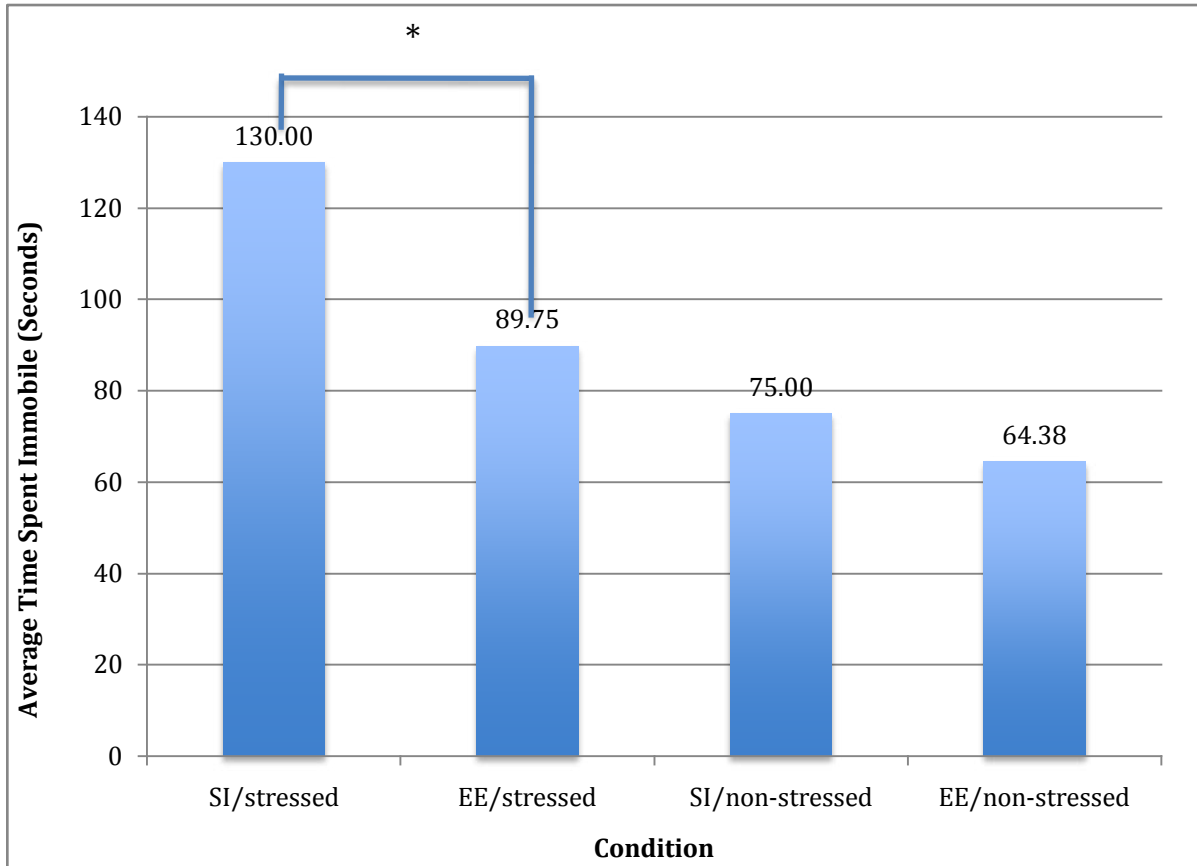
There was no difference in time spent immobile between testing during week 1 and testing during week 2, $F(1) = 3.345$, $p = 0.082$, and no interaction between testing sessions and the four conditions, $F(3) = 0.615$, $p = 0.613$.

There was a significant effect of housing condition on time spent immobile, $F(3) = 20.469$, $p = .000$. A post hoc LSD test revealed that the EE significantly affected immobility in the forced swimming test (see Figure 1). Rats in the SI/stressed condition spent significantly

more time immobile than rats in the EE/stressed condition, $p = .000$, as well as rats in both the SI/non-stressed condition $p = 0.000$, and rats in the EE/non-stressed condition $p = 0.000$. Rats in the EE/non-stressed condition spent slightly less time immobile than rats in the SI/non stressed condition, but this difference was not significant $p = 0.242$ (see Table 1).

Figure 1. Average Time Spent Immobile in the Forced Swim Test.

This figure demonstrates that rats in the EE/stressed conditions spent significantly less time immobile than rats in SI/stressed conditions and that rats in EE/non-stressed conditions spent slightly less time immobile than rats in SI/non-stressed conditions although this difference was not significant. * Indicates significant difference.



Sucrose Preference Test

The ratio of sucrose solution to total consumption of both water and sucrose consumed by each rat was analyzed. Data from each of the two sessions of behavioral testing were used. Total liquid consumption was similar for all rats.

Table 3. Average sucrose consumption in SPT.

Condition	Average sucrose consumption (Standard Deviation)
Testing during week 1	
SI/stressed	0.82 (0.05)
EE/stressed	0.84 (0.12)
SI/non-stressed	0.90 (0.04)
EE/non-stressed	0.89 (0.07)
Testing during week 2	
SI/stressed	0.76 (0.07)
EE/stressed	0.81 (0.05)
SI/non-stressed	0.92 (0.04)
EE/non-stressed	0.90 (0.06)

There was no difference in sucrose consumption between the testing during week 1 and testing during week 2, $F(1) = .543$, $p = 0.469$ and no interaction between the four conditions and the first and second sets of data, $F(3) = .970$ $p = 0.425$.

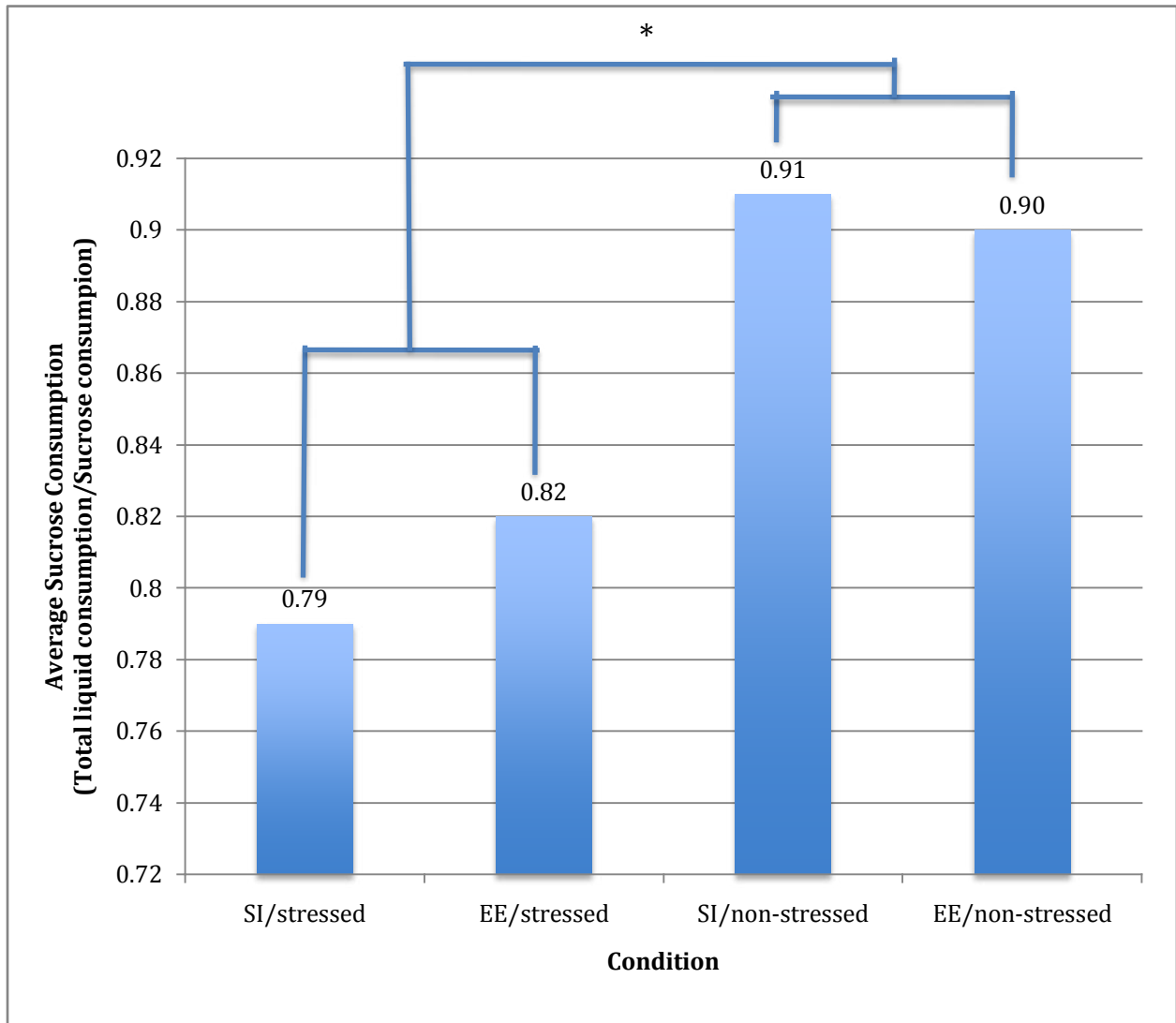
There was however an overall effect of condition on sucrose consumption, $F(3) = 7.049$, $p = 0.002$. An LSD post hoc test revealed that rats in both stress conditions consumed significantly less sucrose than in rats in the counterpart non-stressed conditions (see Figure 2). SI/stressed animals consumed significantly less than both SI/non-stressed animals, $p = 0.001$,

and EE/non-stressed animals, $p = 0.002$. EE/stressed animals also consumed less than SI/non-stressed animals, $p = 0.010$, and EE/non-stressed animals, $p = .020$. When data from both SI and EE conditions were combined there was a significant difference in sucrose consumption between stressed and non-stressed conditions, $t(23) = -4.556$, $p = .000$ (see Figure 2).

EE did not significantly effect sucrose consumption in the stressed conditions. In EE/stressed vs. SI/stressed conditions there was a tendency for EE rats to consume slightly more sucrose solution, but this difference was not significant $p = 0.337$ (see Table 2).

Figure 2. Average Sucrose Consumption In Sucrose Preference Test

This figure demonstrates that rats in both stressed conditions consumed significantly less sucrose than rats in both EE conditions. * Indicates significant difference.



Neurogenesis

A t-test did not reveal a significant difference in neuronal density for the entire dentate gyrus between EE/non-stressed rats and SI/stressed rats, $t(4) = -2.236$, $p = .081$, $d = .78$. In order to examine possible regional differences in neuronal density throughout the dentate gyrus, a one-way ANOVA was used to analyze cell counts region by region and revealed a significant effect of condition in the posterior portion of the dentate gyrus. There was no difference in counts from the anterior region, $F(1) = 4.645$, $p = .097$, $d = .51$ and no difference in counts from the middle region, $F(1) = .852$, $p = .408$, $d = .31$. However, rats from the EE/non-stressed condition demonstrated a higher density of neurons in the posterior portion of the dentate gyrus than rats in the SI/stressed condition $F(1) = 14.087$, $p = .020$, $d = 1.57$ (see Figure 3).

Figure 3. Average density of neurons in dentate gyrus.

This figure demonstrates the density of neurons in the anterior, middle and posterior regions of the hippocampus.

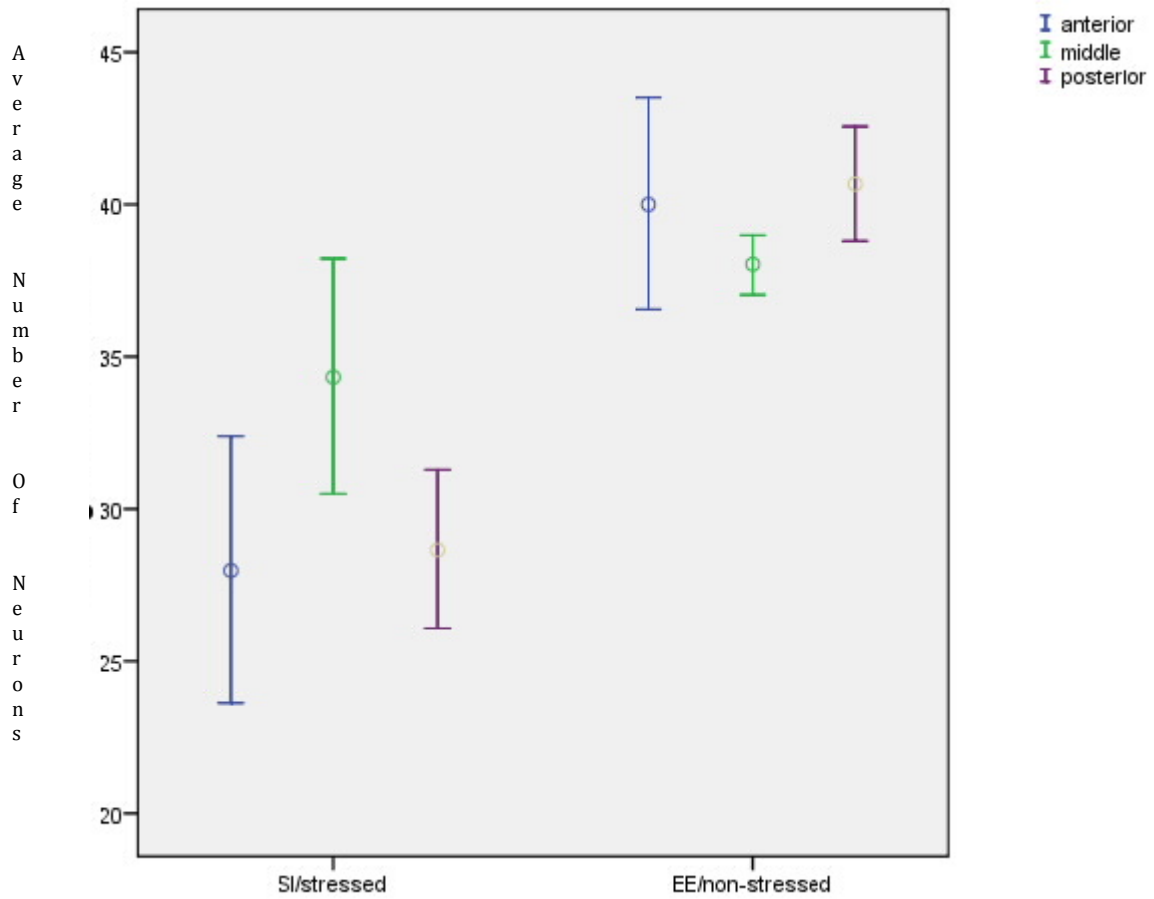


Figure 4. Dentate gyrus of rat housed in SI.

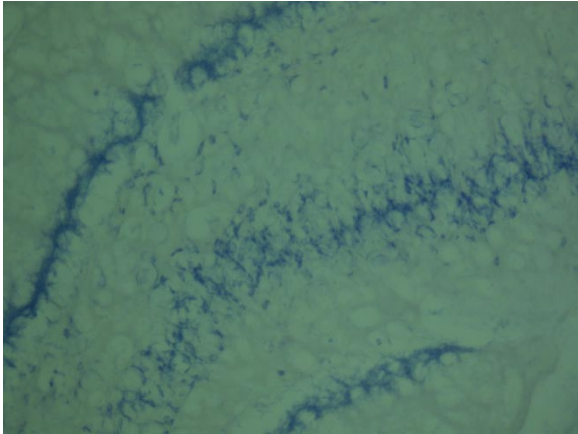


Figure 5. Dentate gyrus of a rat housed in EE.

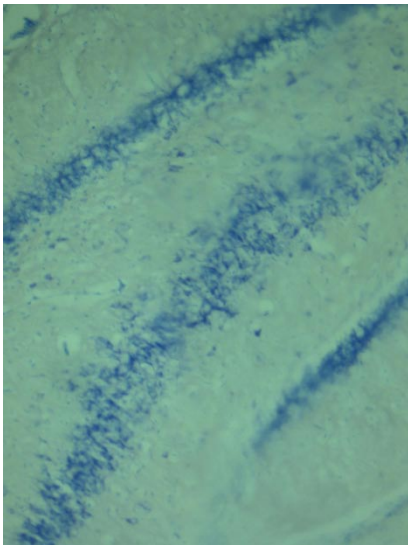


Figure 6. Neurons in the dentate gyrus of a rat housed in SI conditions, after exposure to CMS.

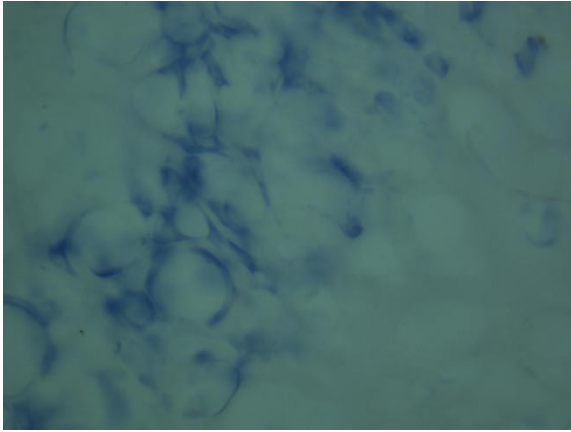
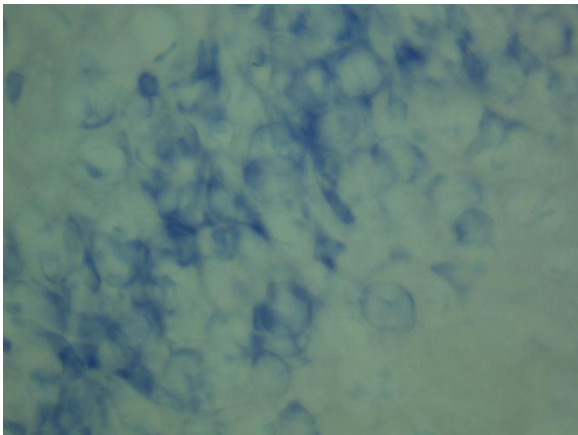


Figure 7. Neurons in the dentate gyrus of a rat housed in EE, after exposure to CMS.



Discussion

These findings support the hypothesis that EE prevents some behavioral symptoms of chronic stress-induced depression. Compared to SI rats that were stressed, EE rats that were stressed spent significantly less time immobile in the FST (see Figure 1), indicating reduced behavioral despair in the EE rats. In a previous study comparing the differential effects of EE and SI on depression related behavior, EE promoted beneficial coping behaviors such as diving and climbing and prevented the development of behavioral despair while SI had the opposite effects (Sanez et al., 2006). Immobility in the FST has such high predictive validity that if a treatment reduces immobility it is classified as having an antidepressant effect (Cryan et al., 2002). Thus, the reduced immobility of rats exposed to EE in this study is strongly indicative of resilience to depression. Coping behavior and reduced immobility in the FST are consistently demonstrated as an effect of rearing in EE (Hattori et al., 2007), but it has never been demonstrated that reduced immobility is maintained even after exposure to a CMS procedure. The present findings demonstrate that the protective effect of EE against behavioral despair persist even through exposure to 14 days of CMS. Contrary to findings of other studies, there were no significant differences in time spent immobile between SI and EE rats that were not stressed. One possible interpretation of this finding is that the protective effect of EE against behavioral despair is stronger in the presence of stress.

The results of the SPT demonstrate that chronic stress was perceived as stressful because regardless of housing conditions, rats exposed to CMS consumed significantly less sucrose than rats that were not exposed to CMS, indicating anhedonia (see Figure 2). There was not a significant difference in sucrose consumption between rats in EE/stressed and EE/non-stressed rats. It is possible that EE does not protect against anhedonia, but sucrose consumption *trends* did support the hypothesis that rats reared in EE consume more sucrose than rats reared in SI after exposure to CMS although these trends were not significant (see Figure 2). This result could be attributed to the specific procedure used in the SPT conducted in this study. Some research indicates unreliable results on the SPT in different strains of rats and with different concentrations of sucrose used in the test. Furthermore, differences in hedonic behavior measured by the SPT have also been attributed to differences in stress protocols used to induce depression (Pothion, Bizot, Trovero, & Belzung, 2004). Further research must be conducted to determine if enrichment does prevent changes in hedonic behavior in response to stress.

The findings of this study also confirmed an increase in neurogenesis in at least part of the dentate gyrus. Notably, a significant difference between rats in the EE/non-stressed and SI/stress group was detected in cell counts from the posterior portion of the dentate gyrus. There was also a trend toward a significant difference in cell counts from the anterior portion of the dentate gyrus with a moderate effect size, $d = .51$. Further research addressing regional differences in neuronal density must be conducted to determine if neuronal density differs by region. In spite of the fact that the global differences did not reach significance, the fact that

the effect size was moderate, $d = .78$, suggests that there was nevertheless an important difference between groups that might have been significant with increased power. Previous research widely confirms that EE increases neurogenesis in the dentate gyrus of the hippocampus (Jungerman, Laroche, & Rampon, 2006; Jayatissa et al., 2006). More specifically, the two most important factors of EE that independently and specifically promote neurogenesis are learning and spontaneous physical activity (Brown, et al., 2003). Similar to embryonic development, adult neurogenesis originates from progenitor cells in the dentate gyrus that migrate to the rest of the hippocampal formation and are functionally integrated into appropriate neural circuits (van praag, et al, 2000). These progenitor cells are susceptible to apoptosis, but rearing in EE promotes the survival of these cells (Hattori, et al., 2007). Conducive to increased neurogenesis, rearing in EE conditions also produces a decrease in corticosterone response to a variety of stressors (Belz, Kennell, Czambel, Rubin & Rhodes, 2003), increases the number of dendritic spines (Berman, Hannigan, Sperry & Zahjac, 1996), and promotes dendritic arborization (Faherty, Kerley & Smeyne, 2003). Rats reared in EE also show increased levels of brain derived neurotrophic factor (Pham, Winblad, Granholm & Mohammed, 2002) . Coupled with an increase in neurogenesis, it is possible that these mechanisms protect the hippocampus from the deleterious effects of chronic stress, producing a physiological buffer against depression.

In this study, during day 1-21 rats housed in EE conditions were maintained under continuous illumination. Subsequently, reversal of light dark cycles was one of the stressors used in the CMS procedure. Reversal of light/dark cycles leads to alterations in rat's behavioral

patterns such as exploratory, eating and drinking behavior, hormone levels and circadian rhythm. Thus, rats undergoing unpredictable shifting of light/dark cycles are exposed to multiple changes, which make this shifting a stressful procedure (Stott, 1981). However, in light/dark cycle shifting, the stress results from the continuous shifting. In experiments where rats are housed under continuous illumination, reestablishment of normal behavior was occurs after 7-9 days (Zucker, 1971). Continuous illumination is initially stressful, but homeostasis is eventually reached. Furthermore, research on mice has found that continuous exposure to light (for 35 days) did not modify anxiety parameters (Castro, et al., 2005). In the present study, it is possible that rats in EE underwent minor stress due to this initial lighting, but this stress is most likely negligible because it occurred early on in the experiment and rats likely adjusted to the lighting conditions. In any case, because EE still had a buffering effect against the CMS procedure, any lighting related stress would imply an even stronger EE effect because EE rats experienced minor stress in the early phase of the experiment and still demonstrated reduced immobility on the FST compared to rats in SI conditions.

The combination of behavioral measures used in this study provides strong evidence that EE can prevent chronic stress induced mood impairments in rats. Just as stress or social isolation are developmental risk factors for depression, the present findings provide strong evidence that EE is a developmental buffer *against* depression. Further research in this area is needed to determine if there is a causal relationship between the increased neurogenesis and the behavioral effects of EE, and to further characterize preventative effects of EE, but the present study provides initial evidence that EE produces a behavioral buffer against depression.

This research is crucial to the discussion of EE because the results show that EE has a preventative influence on depression related behavior both behaviorally and physiologically. Previous research has also demonstrated protective effects of EE in other behavioral measures involving the hippocampus. Housing rats in EE in adulthood prevents memory deficits following both acute stress and postnatal chronic stress (Wright & Conrad, 2008). EE also protects against the behavioral and physiological effects of lead induced neurotoxicity, which causes a reduction in the size and mass of the hippocampus as well as delayed dendritic arborization and NDMA receptor dysfunction (Schneider, Lee, Anderson, Zuck & Lidsky, 2000). The implications of these results are of tremendous importance because they provide a possible model for the prevention of depression related behavior without pharmacological intervention.

In humans, research efforts that have been focused on psychosocial interventions are important because they may reduce the risk of onset and minimize the need for ongoing treatment (Hollon, DeRubeis & Seligman, 1992). For example, intervention programs that focus on the enhancement of cognitive and academic functioning have been successful in promoting positive effects on socio-emotional adjustment, delinquency, and crime in childhood, adolescence, and adulthood (McLaughlin, Campbell, Pungello, & Skinner, 2007). Specifically, in Chicago, Child-Parent Center programs were implemented in the inner city to provide academic and social enrichment for children living in poverty. Researchers found that involvement in these programs produces a protective effect in children that is stronger than risk factors for mood and conduct disorders. Participants in the Child-Parent center had significantly lower rates of adolescent depression, fewer juvenile court petitions, and their probability of

completing high school or their GED was 36% greater than non-participants (Smokowski, Mann, Reynolds, & Fraser, 2004). This example illustrates the importance and apparent success of prevention models in children in at-risk situations.

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