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The Efficacy of Hippocampal Stimulation in Preventing Depressive Symptoms

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THE EFFICACY OF HIPPOCAMPAL STIMULATION IN PREVENTING DEPRESSIVE SYMPTOMS

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ABSTRACT

The hippocampus provides negative feedback for the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis is responsible for producing a response to stressful stimuli. The hippocampus is sensitive to high levels of glucocorticoids (GCs), because of its large number of GC receptors. In times of severe stress, hippocampal function is inhibited and its control over the HPA axis is diminished, leading to hyperactivity of the adrenal glands as well as hypercortisolism, typical of depression. Long-term stress and depression can eventually lead to chronic impairments in cognitive ability, as well as structural damage in the hippocampus. Exercise and environmental enrichment stimulate significant growth and activity in the hippocampus, and have been used successfully as antidepressant treatments in previous studies. However, these previous studies failed to demonstrate whether such treatments are capable of preventing the cognitive symptoms of depression during times of persistent chronic prolonged stress. Previous research has also evaded the possibility of a potential additive effect when both treatments are used in combination. The current study aims to extend previous research in this area by examining both the possibility of a preventative efficacy of hippocampal stimulation during periods of stress, as well the possibility of an additive effect associated with the use of both treatments. Rodents went through a 10-week period of CMS along with concurrent exposure to environmental enrichment, environmental enrichment and

exercise, or neither. Sucrose consumption was used as a measure of anhedonia at the 8-week point. At the completion of the 10 week CMS period, spatial memory was measured using the Morris Water Maze and a Novel Object Placement Task. The overall level of spatial memory impairment was determined based on the group means collected during these tests. Overall, results from the current study provide evidence supporting the preventative efficacy of hippocampal stimulation during periods of stress. While environmental enrichment appeared to be insufficient in preventing the cognitive impairments associated with higher levels of stress, an additive effect of both exercise and enrichment was observed. While it remains unclear whether exercise alone is capable of providing the level of protection observed in this study, the results reveal that exercise is a requisite for the maintenance of hippocampal function in the presence of consistent stress.

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TABLE OF ACRONYMS

ANOVA	Analysis of Variance
BDNF	Brain-Derived Neurotrophic Factor
GC	Glucocorticoids
CMS	Chronic Mild Stress
EE	Environmental Enrichment
HPA axis	Hypothalamic/Pituitary/Adrenal Axis
5-HT_1	Subfamily of Serotonin Receptors
MWM	Morris Water Maze
NOPT	Novel Object Placement Test

CHAPTER I

INTRODUCTION

1.1 STRESS AND THE HIPPOCAMPUS

Chronic stress and depression involve abnormal increases in the manufacturing and release of adrenal hormones known as glucocorticoids or GCs. The hippocampus is a brain structure located in the limbic system; its known primary functions involve the consolidation of declarative and spatial memory. The hippocampus is highly concentrated with receptors for glucocorticoids as it provides the Hypothalamic-Pituitary-Adrenal (HPA) axis with negative feedback regarding the level of GCs in the bloodstream (Stokes, 1995). The HPA axis refers to the structures involved in the manufacturing of the stereotypical stress response. A fully functional hippocampus receiving large quantities of GCs will work to slow the production of these hormones. By providing negative feedback to the HPA axis, the hippocampus is able to exercise inhibitory control over the intensity of the response. These GCs, however, have a damaging effect not only to the structure of the hippocampus, but also on its functioning. Reductions in overall size and cell count occur, as well as impairments in specific types of memory from long-term

exposure to stress. The hippocampus, after significant reductions in size and function, soon becomes unable to provide reliable feedback to the HPA axis, thus leading to the hyperactive stress response system, typical of those who are depressed. While stress slows the growth or function of cells in the hippocampus and leads to cognitive impairment, antidepressant treatments typically promote cell growth and neurogenesis in the hippocampus, while also normalizing cognitive function. Therefore, it is apparent that some relationship exists between mood disorders, such as depression, and the functional state of the hippocampus. The direction of the relationship implies that basic hippocampal maintenance may be an effective step towards preventing the onset of depression. If this is in fact the case, then we would expect to see significant improvements in those who engage in behaviors that promote activity, and increase chemical production in the hippocampus. Indeed, exercise, as well as exposure to enriched surroundings, increases the activity of the hippocampus, and both behavioral treatments have been used effectively in treating depression and its symptoms.

Although these behavioral treatments are successful in repairing the neurological and cognitive damage that occurs as a result of chronic stress and depression, the overwhelming majority of studies introduce these behavioral treatments *after the stressful period has concluded*. Therefore, such studies are demonstrating that exercise and environmental enrichments (EEs) are capable of cognitive repair only when stress is completely absent. Unfortunately, stress is rarely ever removed from one's life entirely and replaced with something beneficial like exercise. A balance between the two is a more plausible real-life scenario. Wright and Conrad (2008) observed that environmental enrichment was capable of preserving hippocampal-mediated function when provided

during a 3-week period of restraint-induced stress. Rodents in this study demonstrated less impairment in spatial memory ability as a result of the concurrent exposure to environmental enrichment. However, the type and duration of the stress treatment was consistent throughout this study allowing for possible habituation during the treatment phase. Also, the enrichment used in the Wright study was non-specific and utilized social as well as environmental and physical stimulation. Therefore, it is uncertain as to which element of their treatment was most efficacious in terms of providing the level of stimulation necessary to diminish the cognitive symptoms of stress.

It remains unclear which specific treatments are sufficiently strong to *prevent* the development of depressive symptoms throughout an *extended period of chronic stress*. The goals of the present study were to determine whether an enriched environment alone, an enriched environment combined with exercise, or both could provide a level of hippocampal stimulation necessary for the prevention of depressive symptoms during a 10-week period of daily chronic mild stress. By providing both stress and behavioral treatments concurrently over an extended period, it was possible to more accurately examine the beneficial and potentially protective qualities of both exercise and environmental enrichment. It was proposed that exercise and an enriched environment, in combination, would not only provide the optimum level of hippocampal stimulation, but would also supply the degree of activity necessary to attenuate the cognitive symptoms of chronic stress. If hippocampal stimulation is necessary for the prevention of these symptoms, then it follows that those treatments providing the hippocampus with the optimum amount of stimulation would be most effective. Therefore, it was predicted that rodents who are exposed to more forms of hippocampal stimulation would experience

observably greater levels of cognitive preservation.

Stress and depression are intimately connected physio-emotional states that have distinct short- and long-term effects on the mind and body. While stress itself is not considered a form of depression, depression is typically accompanied by a significant amount of stress. Stressful life events that occur, independent of an individual's behavior, substantially increase the risk of experiencing a depressive episode. A causal relationship is believed to exist between chronic stress and the later development of depressive symptoms (Kendler, Karkowski, & Prescott, 1999).

Depression often results in notable changes in cognitive function (typically impairments, but, as we will discuss, not always). Attention, memory, visuomotor speed and language can all be negatively affected. Furthermore, both stress and depression can lead to atrophy and cell loss in the hippocampus (Duman, 2004; Ravnkilde, et al., 2002). In fact, higher levels of GCs in the blood lead to greater reductions in hippocampal volume (Ohl, et al., 1999). Volume loss is also strongly associated with lower cognitive performance, while higher cognitive performance is associated with lower levels of GCs (Starkman, Giordani, Gebarski & Schteingart, 2003). In other words, stress or exposure to GCs can damage the hippocampus. Cell count is lower in the hippocampus of animals exposed to higher levels of GCs. Slowed cell transmission as well as changes in overall cell structure can occur as a result of greater exposure to stress (Gould & Tanapat 1999; Keenan & Kuhn, 1999; Sandi, et al., 2003; Stein-Behrens et al., 1994; Stewart et al., 2005; Stokes 1995). It is clear how long-term stress and depression lead to cognitive decline. Chronic stress actually inhibits the production of brain-derived neurotrophic factor or BDNF, a growth factor required for neurogenesis and neuroprotection,

essentially a neuronal requisite for learning (Choy, de Visser, Nichols & van den Buuse, 2008; Fabel et al., 2003).

1.1.1 STRESS AND MEMORY

Memory is highly sensitive to stress. Specific memory impairments are consistent with both chronic stress and depression, as GCs have deleterious effects on distinct types of memory (Buchanan, & Tranel, 2008). Interestingly, different forms of memory are altered by stress in different ways. Memory processes are also uniquely affected. Stress facilitates the consolidation of memories, while impairing the recollection of previously stored information (Daimond, Fleshner, Ingersoll & Rose, 1996; Roozendaal, 2002; Kuhlmann, Piel & Wolf, 2005; Beckner, Tucker, Delville & Mohr, 2006; Wolf, 2008; Schoofs, Wolf & Smeets, 2009). A perceivable level of emotional arousal is required for the negative effects of stress on memory retrieval to emerge (Tollenaar, Elzinga, Spinhoven, & Everaerd, 2008). Conversely, noradrenaline release is critical in facilitating memory consolidation during a stressful experience, such as a flashbulb memory (Roozendaal, McEwen & Chattarji, 2009). Stress can also enhance the strength of implicit memories, such as conditioned responses to fearful stimuli (Sapolsky, 2003). Such memories are more available in those who are depressed, demonstrated by their characteristic automatic memory biases towards negative information (Bradley, Mogg & Williams, 1995).

Working memory is also impaired during periods of stress, with higher stress being associated with lower performance (Oei, et al., 2006; Schoofs, Wolf & Smeets, 2009). However, it is hippocampal dependent memory that experiences the greatest deficits

during times of heightened stress (Ohl, et al., 1999). High cortisol levels negatively affect general declarative memory retrieval (Kirschbaum et al., 1996; Buchanan & Tranel, 2008). Stressed participants demonstrate the impaired retrieval of autobiographical and socially relevant memories (Rosch, 1997; Buss, Wolf, Witt & Hellhammer, 2004; Merz, Wolf & Hennig, 2010). In addition, the recognition of novelty is impaired in rodents who are under stress. (Elizalde, et al., 2008).

The hippocampus is also the primary structure involved in the consolidation and retrieval of spatial memory. It contains place cells that fire in accordance with specific objects in the environment, leading to familiarization of different areas and places. The hippocampus essentially acts as a map that stores information regarding visited locations. This type of memory, known as spatial memory, is also considerably impaired by increases in stress (Kirschbaum, et al., 1996; Keenan & Kuhn, 1999; Hu, Xuemei, Shengwang & Changlin, 2003; Song, et al., 2006; Moosavi, Naghdi, Maghsoudi & Zahedi, 2007). It has been suggested that spatial memory deficits occur as a result of the harmful effects of stress on place cell firing stability (Kim et al., 2007). If these cells do not fire correctly in accordance with a familiar environment, then this environment will appear less recognizable.

During chronic stress and depression, hippocampal neurogenesis is reduced and cell loss and atrophy are observable. These developmental deficits lead to noticeable impairments in cognitive performance that are strongly associated with higher cortisol levels and lower hippocampal volume (Starkman, Giordani, Gebarski & Scteingart, 2003).

1.2 REVERSAL OF DEPRESSIVE SYMPTOMS

Fortunately, these symptoms are in no way permanent. Researchers have found that chronic antidepressant treatment is successful in reversing the course of depressive symptoms, specifically in the hippocampus (Burt, Niederehe & Zembar, 1995; McEwen, 2000; Czeh & Lucassen, 2007). The hippocampus then appears to be the main structure of interest when measuring the damaging effects of stress, as well as the favorable results of antidepressant treatment. It has been proposed that regulation of neurogenesis in the hippocampus is critical in the treatment of depression (Duman, 2004; Becker & Wojtomicz, 2006). It is believed that human and rodent hippocampi are analogous, since damage to both produces corresponding behavioral impairments (Goodrich-Hunsaker, Livingstone, Skelton & Hopkins, 2010). A parallel between the functions of the hippocampus in both humans and rodents is imperative. The results of this study can only be generalized to humans if the structures are comparable between the two species. Specifically, the hippocampi of rats and humans have generally been found to mediate similar if not identical functions. These include spatial and temporal pattern separation, sequential learning, spatial and temporal pattern associations, spatial and temporal pattern completion, and short-term and intermediate-term memory (Kesner & Hopkins, 2006).

1.2.1 ENVIRONMENTAL ENRICHMENT

Environmental enrichment involves long-term exposure to novel surroundings. Enriched environments (EEs), unlike exercise, provide novel sensory stimulation and numerous opportunities for learning and manipulation. Housing in an enriched environment has notable beneficial effects on the hippocampal structure and function of

rodents. Age-related impairments in spatial memory are reduced after exposure to EEs (Anisman, Zaharia, Meaney & Merali, 1998; Nilsson, 1999; Lores-Arnaiz, et al, 2006; Wright & Conrad, 2008; Frick, Stearns, Pan & Berger-Sweeney, 2010). These improvements are the product of increased neurogenesis, increases in levels of nerve growth factors (such as BDNF), noradrenaline, as well as 5-HT1 production and transmission in the hippocampus following exposure to EEs (Naka, shiga, Yaguchi & Okado, 2001; Rasmuson, et al., 1997; Rosenzweig & Bennett, 1996; Torasdotter, et al., 1998). 5-HT1 is a subfamily of seretonin (5-HT) receptors that when activated, inhibit the response of the sympathetic nervous system, thereby dulling the intensity of the sympathetic response to stressful stimuli.

In animal models of depression, enriched environments appear to have an antidepressant-like effect on rodents (Brenes, Rodriguez & Fornaguera, 2006). Behaviorally, chronically stressed rodents housed in enriched environments show less escape-oriented, and more exploratory behaviors in novel situations than do stressed rodents housed in standard laboratory conditions (Larsson, Winblad & Mohammed, 2002). Non-stressed rodents housed in EEs also demonstrate more rapid habituation to novelty than controls, as well as larger reductions in their startle response (Hattori, et al., 2007). It has been suggested that housing in an EE improves an animal's information–processing ability, allowing for more efficient learning and responses to novelty consistent with the notion that exposure to EE results in functional preservation of the hippocampus (Brenes, Rodriguez & Fornaguera, 2007). Novel situations would be placed in the proper context based on previously stored information, allowing for more appropriate physio-behavioral responses to unfamiliar stressors (Becker & Wojtomicz,

2006).

1.2.2 EXERCISE

Physical activity, like EEs, also improves learning and memory in humans and animals (Van Praag, 2009). Both exercise and environmental enrichment provide protection against age-related memory impairments (O'Callaghan, Griffin & Kelly, 2009). In fact, the cognitive and emotional benefits of EEs and exercise are highly similar. Similar to EE, exercise leads to improvements in learning and memory, increases in the rate of hippocampal neurogenesis through the heightened production of nerve growth factors in rodents, as well as an increase in 5-HT1 production (Bjornebekk, Mathe & Brene, 2005; Christie et al, 2008; Cotman & Berchtold, 2002; Cotman, Berchtold & Christie, 2007; Grace, Hescham, Kellaway & Bugarith, 2009; O'Callaghan, Ohle & Kelly, 2007; Vaynman, Ying & Gomez-Pinilla, 2004; Winter, et al., 2007).

EEs and exercise are both believed to be capable of promoting new and more proficient types of cognitive processing. Overall brain function is made more efficient through increases in neurotransmitters, nerve growth factors, and synaptic plasticity, allowing for faster processing speeds, and greater cognitive flexibility (Chodzko-Zajko, 1991; Christie et al., 2008; Hillman, Snook & Jerome, 2003).

Most relevant to the current study, exercise like EE, also works as an antidepressant (McCann & Holmes, 1984). In fact, exercise can be considered a more effective anti-depressant than typical anti-depressant medications. Rates of relapse are lower in those who exercise regularly with and without medication. In fact, the rates are the lowest for those who exercise exclusively (Babyak, et al., 2000; Brosse, Sheets, Lett & Blumenthal,

2002). Exercise is also the strongest neurogenic stimulus out of all available anti-depressant treatments (O'Callaghan, Griffin & Kelly, 2009). There does not appear to be a notable dissimilarity in the stimulatory efficacy of aerobic and anaerobic exercise, and combining the two may potentially have an additive effect (Brosse, Sheets, Lett & Blumenthal 2002; Strohle, 2009). Fortunately, individuals who increase their activity over time are at no greater risk for depression than those who have remained active, as the improvements are almost immediately effective. Similarly, wheel running in rodents results in a three-fold increase in the production and survival of new neurons during only the first 32 days of activity, with cell genesis peaking at 3 days. However, those who used to be, but are no longer active are 1.5 times more likely to develop depressive symptoms than those who maintain an active lifestyle, (Babyak, et al, 2000). It is quite possible that this higher rate of depression is due to a lack of hippocampal maintenance provided in the form of exercise.

While it is clear that exercise can be used as an effective treatment for depression, there remains some disagreement over whether or not exercise can prevent the onset of depressive symptoms proactively in the presence of long-term external stress (Greenwood, et al., 2003; Palmer, 2005; Paluska & Schwenk, 2000;). The current study aims to answer this question by examining the neuro-protective efficacy of both exercise and EEs when provided concurrently with long-term chronic stress. By assimilating both EEs and exercise, it will be possible to measure the preventative power of not just EEs, but EEs when combined with the ability to exercise, while stress is consistently present. It is predicted that both treatments used in combination will demonstrate the preventative power to overcome the onset of depressive symptoms throughout the chronic stress

period.

1.3 CHRONIC MILD STRESS

The chronic mild stress model of depression, or CMS, is intended to mimic naturalistic stressors over an extended time period, in order to more reliably produce depressive symptoms in rodents. In the CMS model, rodents are exposed sequentially to a variety of mild stressors such as food or water deprivation once every 10 to 14 hours, for a period of weeks or months. No single stressor is necessary or sufficient to result in any measurable change in the cognitive state of the rodent (Willner, 1997; Willner, 1997). Each stressor on its own is harmless, yet the high frequency, as well as the unpredictability in the manner they are provided, results in the long-term development of depressive symptoms. The stress response is not a static event. The intensity of each response can vary based on the specific nature of the present stressor. Those that are both uncontrollable and contain social-evaluative potential elicit the largest and most prolonged increases in cortisol levels (Dickerson & Kemeny, 2004). The CMS model, being uncontrollable in design, is therefore assumed to be a more reliable technique for producing depressive symptoms than more severe stress treatments of shorter durations.

Exposure to CMS in rodents results in significant reductions in body weight and locomotor activity. Rodents also experience corticosterone hypersecretion, typical of HPA axis hyperactivity, as well as a variety of sleep disorders characteristic of depression (Willner, 1997; Xu, et al, 2008). More importantly however, CMS leads to anhedonic behavior in rats and mice. (Elizalde, et. al., 2008; Enkel, Spanagel, Vollmayr & Schneider, 2010; Grippo, Beltz & Johnson, 2003; Henningsen, et al., 2009; Jans &

Blkland, 2008; Muscat, Papp & Willner, 1992; Willner, 1997; Pohl, et al., 2007; Xu et al., 2008).

Anhedonia is defined as a decreased interest in pleasure in almost all aspects of life, likely associated with a decrease in the production of dopamine common among depressed individuals (Muscat, Papp & Willner, 1992). Anhedonia is the principal distinguishing feature of depression in rodents (Pohl, et al., 2007). Over 70% of rats that undergo CMS treatment demonstrate anhedonic-like behavior, in the form of reduced levels of sucrose intake (Henningsen, et al., 2009). During or after the stress treatment, rodents are given a palatable solution of sucrose and water. The ability to enjoy the sweetness is inferred by measuring the amount consumed. Smaller amounts presumably indicate lower sensitivity for reward, or anhedonia. Normal water consumption remains unaffected by CMS (Muscatt, Papp & Willner, 1992; Willner, 1997). Anhedonia, much like the other symptoms of chronic stress and depression, is reversible with the use of anti-depressants (Muscat, Papp & Willner, 1992; Willner, 1997; Elizalde, et al., 2008). Chronic anti-depressant treatment has even been shown to prevent the development of anhedonia in rodents exposed to CMS (Grippo, Beltz, Weiss & Johnson, 2006).

1.4 TREATMENT AND MEASUREMENTS OF SYMPTOMS

Anhedonic behavior, spatial memory ability, and novel object and novel placement recognition are the standard quantitative measures of depression in rodents. The current study utilized these measures in order to more thoroughly examine the severity of the depressive symptoms induced by 10 weeks of CMS. Previous studies using CMS as a model of depression have experienced visible symptoms after only four weeks of

application (Grippo, Beltz & Johnson, 2003; Jans & Blokland, 2008). Providing 10 weeks of CMS minimized the risk of a delayed response to the stress and also maximized the depressive capability of the treatment. Pairing EEs and exercise treatments with an extended, concurrent period of CMS allowed the researchers to more accurately measure their preventative capacity, rather than their influence in treatment.

Other related studies typically removed the stressor prior to the introduction of the anti-depressant, which fails to accurately mimic the normal fluctuations of traditional stress in non-experimental environments. This study also compared the protective potential of exposure to EEs with and without access to physical activity, which to our knowledge has not been done previously.

The current study proposed to take a step toward answering questions involving the ability of treatments that promote significant hippocampal activity to attenuate the severity of depressive symptoms when those treatments are administered during CMS. Several aspects of our daily life have the potential to alter our brain health and cognitive function (Gomez-Pinilla, 2008). Consistent physical activity, as well as prolonged exposure to EEs both lead to the reversal of stress-related damage to the hippocampus and were therefore used as manipulations in the current study.

Based on these observations, the present study intended to focus on the hippocampus, and the behavioral maintenance of this structure, as a means of potentially preventing the onset of depressive symptoms. It was expected that consistent hippocampal stimulation would be effective in preventing the depressive effects of long-term chronic stress. However, a behavioral model of prevention requires identification of specific treatments (manipulations) that have antidepressant properties in terms of

hippocampal growth and preservation.

1.5 RESEARCH QUESTION AND HYPOTHESES

The current study was designed to examine whether consistent hippocampal stimulation was effective in preventing the long-term cognitive symptoms of chronic stress and depression. The study also intended to isolate the two treatments (environmental enrichment and enrichment plus exercise) in order to identify whether an enriched environment was sufficient in preventing depressive symptoms or if the inclusion of exercise was necessary.

Hypothesis 1: Rats housed in isolation and spared the CMS treatment (true controls) will not be significantly different in behavior from the CMS group that receives EEs alone, nor different from the CMS group that receives EEs with access to exercise.

Hypothesis 2: Rats placed in EEs with access to a running wheel during exposure to 10 weeks of CMS will display less behavioral impairments than rats in stark environments or those receiving only EEs.

Hypothesis 3: Rats placed in EEs without access to running wheels during the CMS treatment will show less behavioral impairment than stressed rats in stark environments.

These hypotheses were tested by pursuing the following specific aims:

Aim 1: Divide the experimental groups in terms of behavioral treatments while keeping the CMS procedure consistent among all groups except true controls. Ensure that the difference in behavioral impairment is due solely to the specific treatments that separate the 4 groups.

Aim 2: Quantify the extent of depressive symptoms following 8 weeks of CMS in three different experimental groups by measuring their level of anhedonia in comparison with the control group using the sucrose drinking test.

Aim 3: Measure and compare the behavioral impairment of all four groups using their performance in the Morris water maze and Novel Object Placement Test that in addition to memory, examine:

1. General locomotor activity
2. Sensory/motor processing (ability to see and use spatial cues for navigational purposes)
3. Assessment of rewarding stimuli (willingness to escape the water)

CHAPTER II

MATERIALS AND METHODS

2.1 RESEARCH DESIGN

In this true experimental design concurrent behavioral stimulation and CMS in individually housed rodents.

Rats were assigned to one of four experimental groups:

1. Control,
2. Stress only
3. Stress/EEs
4. Stress/EEs/Exercise.

Dependent upon group assignment rats received either EEs in the form of cage toys, exercise and EEs, or neither exercise nor EEs (see Table 1). All groups, with the exception of the control group, underwent 10 weeks of CMS that was provided concurrently with their assigned behavioral treatment. The differences in the preventative quality of the treatments were measured based on the results of behavioral tests designed

Table I.

***Descriptions of the Treatment Levels*.** Treatments were defined by the administration or withholding of stress as well as the exposure or lack thereof to enrichment in the form of toys, natural food, and exercise.

GROUPS	Controls	Stark Environment	EEs	EEs/Exercise
MANIPULATIONS				
Chronic Mild Stress		✓	✓	✓
EEs (Environmental/ Dietary)			✓	✓
Exercise				✓
INSTRUMENTS				
Sucrose-Drinking Test	✓	✓	✓	✓
Water Maze Test	✓	✓	✓	✓
Novel Object Recognition Test	✓	✓	✓	✓

to test abilities normally impaired by chronic stress and depression.

2.2 TIMELINE OF TREATMENTS AND TESTS

Days 1-3: Acclimatization period (No stress). Newly arriving rats were handled for 2 minutes per day for the first 3 days in laboratory.

Days 4-56: First 8 weeks of CMS treatment**.**

Days 56-60: Sucrose test was given to determine the severity of anhedonia among individual rats thus far. The CMS continued throughout.

Days 60-70: CMS continued for the last two weeks until completion of all behavioral testing.

Days 70-72: Morris Water maze testing began in order to measure the spatial memory abilities of each rodent. The water maze test concluded on the third day with a probe test.

Days 72-74: CMS continued.

Day 74: Novel Object Placement Task was given in order to measure object placement recognition ability.

2.3 SUBJECTS

Twenty-four male Long Evans rats aged between 45-49 days old at time of arrival. Rats were ordered from Harlan Labs in Indianapolis, IN. The rats were randomly placed in one of the four experimental groups at the time of arrival. They were then housed individually except when grouping was required as a stressor. Food and water were available ad libitum, except during periods of deprivation. The temperature in the laboratory was kept constant at 73° F or 23° C. There was a standard 12 hour

light/dark cycle from 7 a.m. to 7 p.m. excluding the stress periods involving variations in illumination. This study was approved by the Cleveland State Institutional Animal Care and use Committee to ensure the ethical treatment of laboratory animals in research.

2.4 MATERIALS

The 24 rats were housed in 24 separate individual plastic cages measuring 21”x 15 ½”x 8” with stainless steel lids. Each cage contained easily accessible food and water as well as sufficient bedding. The cages in the EEs group were provided with items such as plastic balls, huts and plastic or cardboard tubes and chewable toys. Toys were alternated with others after a few days in order to maintain consistent novelty. The cages in the EEs/exercise group contained running wheels (Large Flying Saucer Wheel, Ware Manufacturing Inc. USA) as well as enrichment toys. The platform for the wheels was secured to the bottom of the cages so as to minimize any difficulties in maintaining the availability of exercise. The groups receiving EEs were also provided with natural dietary supplementation in the form of fruits, nuts or vegetables three days a week so as to provide multiple forms of sensory stimulation. These food items were supplied in addition to their regular diet of 18% protein rat pellets. Eighteen wire cage lids were employed as wedges to tilt the cages 45°. Two 75 Watt (Chauvet®Lighting, USA) strobe lights attached to a light stand were used during the stroboscopic light stress period. The exercise/EEs group’s exercise wheels held a small magnet that triggered the magnetic switches attached to the side of the cage. These switches then communicated with a Mini-Counter (Columbus Instruments, USA) that reported to a PC that records the total number of revolutions ran per rat per day.

2.4.1 SUCROSE CONSUMPTION TEST

Sucrose solution (450 ml at 1.5%) was available in a labeled water bottle for exactly four days (96 hours).

2.4.2 MORRIS WATER MAZE

Rodents were moved to smaller cages containing no bedding prior to being transported to the water maze room in order to preserve the aridity of the original bedding. A 6'(183 cm) in diameter and 2' (61 cm) deep round galvanized metal pool was used for the maze. Construction paper geometric figures were taped to the inside of the pool to act as visual reference cues. A hidden platform 6" (15 cm) in diameter was placed ¾" (2 cm) below water level in a designated quarter of the tank where it remained for the entirety of the testing. Non-toxic, white tempura paint (Sargent Art, USA) was used to cloud the water and mask the location of the platform. The movements of the rat were tracked by a video surveillance camera model number XAVEE-B480AC-D/N (Xavee, USA Distributor) the ceiling directly above the pool. The camera communicated with the Videomex Water Maze System V.5 software (Columbus Instruments, USA) loaded onto the HP Vectra 466 PC. This software measured the latency to find the platform as well as total distance traveled and the rodent's overall proximity to the platform.

2.4.3 NOVEL OBJECT PLACEMENT TASK

The same pool used in the water maze was used for the Novel Object Placement Task. The visual cues remained in place as they should have little impact on the outcome

of the test. Two novel toys were placed inside the empty, dry pool. The test was not only tracked using the Videomex software, but it was also documented on a DVD, using Panasonic DVD recorder model DMR-EZ48VK that was connected to the surveillance camera.

2.5 PROCEDURES

A slight variation of the protocol developed by Papp (1991) was used. The stress protocol consisted of eight different stressors: one period of intermittent illumination stroboscopic light, 45° cage tilting, paired housing (up to two hours), two periods of food or water deprivation, food *and* water deprivation, soiled cage (wet bedding), and no stress. Rats were housed individually and each underwent a three day period of acclimatization prior to any stress exposure. During this period, each rat was handled for two minutes a day in order to prepare them for later handling. The rats were then placed back into their cages until the next day. On the first day of the CMS treatment, the first stressor was applied in the morning. Each stressor, with the exception of the paired housing, lasted 10-14 consecutive hours. Therefore, stressors were most often applied in the morning and then again later in the evening. The CMS treatment continued on for 10 full weeks. For the EEs and the EEs/exercise groups, novel food items were provided to supplement their normal diet. Small amounts of natural foods such as fruits, vegetables or nuts were given as additional enrichment three days a week throughout the 10-week period.

The CMS method consisted of eight stressors applied 12 times a week along with two periods of no stress (see Table II). The order of application of these stressors was

semi-random. Stroboscopic light is most effective in the dark therefore its use was limited to the evening. Also, food and water deprivation, though available twice each week were not used consecutively so as to maintain mild levels of dietary stress. The CMS treatment was administered for a 10-week period and throughout the final week of behavioral testing.

Table II.

***List of Weekly Stressors*.** The list of the type of stressors, their duration as well as the frequency of their administration each week.

Weekly Schedule:

Stressor	# of periods	Information
Soiled (wet) Caging	2	250-400 ml of water poured into bedding
No food or water	2	Access to food or water denied
No food *and* water	2	Access to food and water removed
No stress	2	All forms of stress removed
Stroboscopic light	1	Two Strobe lights set at 150 flashes/ min.
Paired housing	1	Rats paired in cages for up to 2 hours
Intermittent Illumination	1	Lights are turned off/on every two hours
45° Cage tilt	1	Cages are tilted at a 45° angle.

2.5.1 WEIGHT

Each rat was weighed at the completion of the acclimatization period in order to record their starting weight. Weights were recorded once a week for the remainder of the experiment as a measure of each rats developmental health. The mean overall weight fluctuations of the groups, as well as the mean increases in weight per week, were recorded.

2.5.2 SUCROSE CONSUMPTION TEST

Rats were deprived of water for 14 hours prior to the administration of the sucrose test. At the completion of week eight of CMS each rat was given both regular water and 450 ml of a palatable 1.5% sucrose solution. The rats had access to both the solution and their regular water ad libitum for four consecutive days (96 hours). Ball bearing-filled sipper tubes were used for the sucrose solution to minimize leakage. At the completion of testing the remaining solution was measured and subtracted from the original 450ml, leaving the total amount consumed. The amounts consumed were separated by group, and their means were calculated.

2.5.3 RUNNING WHEEL

The rats were allowed to run ad libitum. Small magnets were affixed to the running wheels in the Enrichment/exercise group's cages. These magnets triggered switches attached to the exterior of the cage. Each revolution of the wheel closed the magnetic switch, which was wired to a Mini-Counter that quantified the number of revolutions each rat completed. These numbers were recorded in order to determine

the developmental health of the rats.

2.5.4 MORRIS WATER MAZE

The Morris water maze (Morris, 1984) was specifically designed as a measure of spatial memory ability in rodents. The water maze consisted of a round galvanized metal pool (188.88 cm in diameter, 60.96 cm deep), that contained various geometric visual cues attached to the interior (see Figure 1). The pool was separated into four quadrants. A round platform (20.32cm tall, 15.24cm in diameter) was placed in the same quadrant for every trial with the exception of the probe test. The tank was filled with water (23-29° C) until it reached 2cm above the top of the platform. Once the tank was filled, white tempura paint was added to mask the location of the platform. Rats underwent a two-trial (one block) training period the night prior to the beginning of testing.

Rats were placed in the water at a randomly chosen location around the pool for each block. The rats were lowered into the water facing the inside wall of the pool. Once the rat was in the water the tracking of its movement began. Each trial lasted a maximum of 60 seconds. If the rat was unable to find the platform in less than 60 seconds, it was manually positioned on the platform where it remained for 30 seconds until being removed, toweled off and placed back in its cage. If the rat was successful in locating the platform**,** 30 seconds was also given until removal. Once the first round of trials had been completed the second round of trials began. Rats were placed in the water from the same location as in the previous trial and in the same order. After this training period each rat was tested in five blocks of two trials each

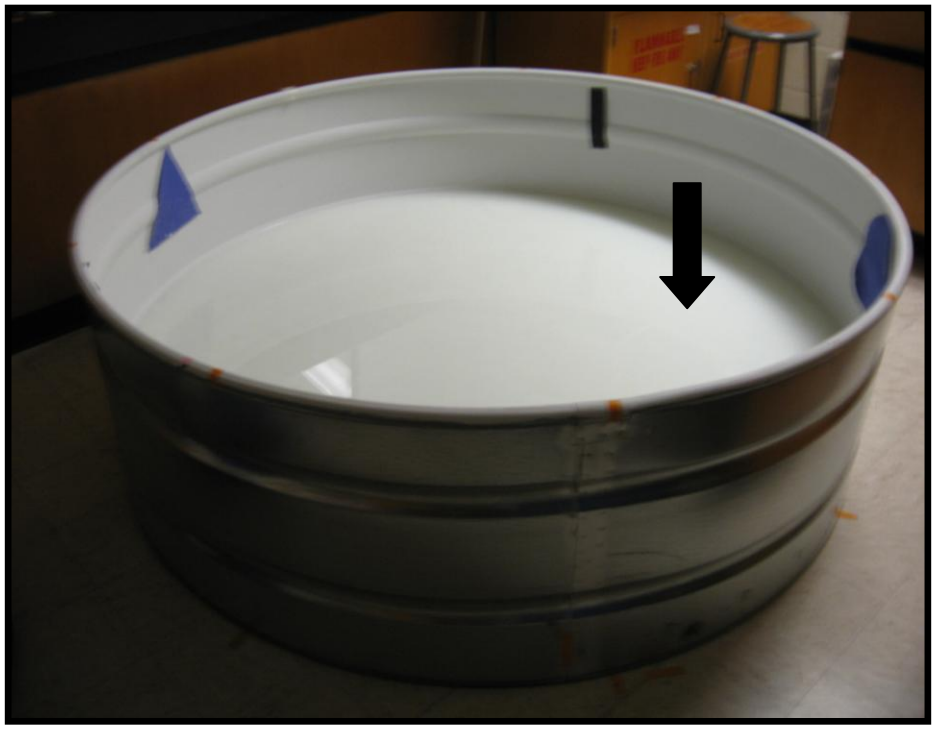

Figure 1. The Morris Water Maze. After the 10th week of CMS, the spatial memory of the rodents was measured based on the rate that they learned the location of a hidden platform over 10 trials. (The platform was located on the right)

(10 total trials) over the next three days. For every trial, the latency to find the platform, total path length and mean proximity to the platform were recorded for each rat, and the overall group means were calculated.

A probe test was administered six hours after the last trial, which examined each rat's retention of the location of the original platform. In the probe test the platform was removed and each rat swam for the maximum 60 seconds without interference. Each group's mean proximity to the platform as well as the total number of crossings into the original location was recorded as measures of spatial memory.

2.5.5 NOVEL OBJECT PLACEMENT TEST

This test was based on the observation by Ennaceur and Delacour (1988) that rodents have a natural inclination to spend more time exploring unfamiliar objects. However, unlike conventional novel object tasks that measure the response to novelty in appearance, this test further examined spatial memory impairment by exploring the response to novelty in location (Li, et al., 2008). Two days after the probe test, the rats were returned to the water maze testing room. The pool was drained of water and dried before testing began. The movements of the rats were tracked with the same Videomex Water Maze System V.5 software located on the HP Vectra 466 PC. The surveillance camera above the pool was also connected to a DVD recorder that was used to record each trial.

The complete test was broken up into two trials labeled trial A and trial B. The location of the objects and entry point in both trials was the same for every rodent. Two entirely novel objects were placed directly in the center of two pool quadrants

approximately 36” (91 cm) apart and 18” (46 cm) from the sides of the pool. These quadrants were side by side and equal distance from the first point of entry, eliminating any potential for proximity-based partiality to either object. The rodents were placed in the pool facing both objects and were given five minutes (300 seconds) to explore. The time spent in each quadrant as well as the time spent in the area directly surrounding each object were recorded. The pool was rinsed or disinfected after each five-minute trial to eliminate any possible olfactory cues.

Once the first trial was completed, the location of one of the objects was altered. The toy that was originally positioned in the top left quadrant relative to the point of entry was moved to the bottom right quadrant of the pool. The point of entry was also moved 90° to the left (see Figure 2). Therefore, the toy that was originally located on the rodent’s left side was now on its right. Once again the rodents were given five minutes to explore while their movements were tracked and recorded. The percentage increase in the time spent exploring the toy before and after it was moved was measured. Also measured was the percent decrease in the proximity to the toy in trial after it had been moved.

2.6 DATA ANALYSIS

Pearson product-moment correlations were performed to examine the relationship between data gathered throughout the treatment period, including weekly weights taken as well as the amount of sucrose consumed over the four day testing period at the end of the 8^{th} week, and the performance in the Morris water maze (MWM). A one-way ANOVA was performed to identify whether there was a significant difference in weight gained between the groups during the first six weeks

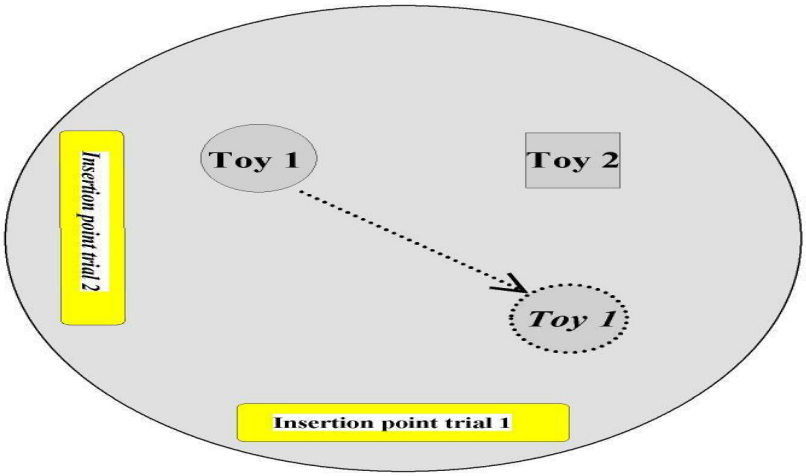

Figure 2. Novel Object Placement Test. The figure shows the point of entry and the position of the novel toys in both trials. This test measured the rodents' awareness of novel location.

of CMS. An ANOVA was also performed to examine the differences between the groups' mean sucrose consumptions. For the MWM, an ANOVA was performed comparing the groups based on the latency to find the platform, the percentage of the total time spent swimming in the platform quadrant, and also the mean path length per group. A significance level of .05 was used. Tukey post-hoc comparisons were used to examine the specific groups that differed.

A strong correlation between either sucrose consumption and testing performance or weight and testing performance would suggest that rodents exhibiting more mild depressive symptoms also performed better on spatial memory tasks. Differences between the experimental groups in mean sucrose consumption and mean weight would suggest variations in the sensitivity of each group to the CMS. Statistical evidence such as this would imply dissimilarity in the overall stress levels of the four groups based on the different treatments provided. The experimental groups that perform most successfully throughout the behavioral testing will be believed to have benefited the most from their specific experimental treatment. There may be alternative reasons for obtaining significant results from these behavioral tests. It is possible that the exercise group may simply become better swimmers and therefore take less time to reach the platform. This can potentially be ruled out as an explanation since learning the location of the platform must be accomplished independent of swimming ability. Therefore, it is the learning and spatial memory ability that allows the rodents to find and preserve the location of the platform rather than the physical development of the rodents.

It is also feasible that it is the enrichment of both the toys and the running wheels that could potentially result in behavioral preservation rather than the specific benefits of

the exercise. Perhaps the running wheel will function more as a source of perceptual enrichment as opposed to a source of exercise. This can potentially be ruled out based on the differences in the outcomes of the two enriched groups. Similar results from both the enriched and the enriched/exercise group would make this distinction difficult. However, if these groups differ from one another significantly, then it can be concluded that it was the distinguishing benefits of the exercise and not simply more enrichment that produced these results.

CHAPTER III

RESULTS

Table III.

Brief Statistical Analysis. "Non-sig" indicates there were no significant differences noted by the analyses. "Sig" indicates at least partial significance noted among the analyses that were conducted. See the appropriate headings of this section for details.

EXPERIMENT	Comparison of Group Means	Sig	Non-sig	Tests of Association	Sig	Non-sig
Body Weights	**ANOVA/Tukey's**	✓		**Group x Mean weight increase**	✓	
Sucrose-Drinking Test	**ANOVA/Tukey's**		✓	**Group x Mean amount consumed**		✓
Water Maze Test	**ANOVA/Tukey's** **RM ANOVA/Tukey's**	✓ ✓		**Group x Mean latency** **Group x Path length**	✓ ✓	
Water Maze Probe	**ANOVA**		✓	**Proximity x Number of Entries (Negative)**	✓	
Novel Object Placement Test	**ANOVA**		✓			✓

3.1 WEIGHTS

Weights were recorded weekly for each participant beginning at the end of the first full week of CMS and continuing until the end of the 6th week (see Figure 3). Due to the variation in the age of the rodents at the time of arrival, mean weight gained per week was utilized, as opposed to the end or weekly weight (see Figure 4). An examination of the first six weeks of weight gain and loss between the groups using ANOVA revealed a significant difference in weight gain beginning in the third week of treatment (F (3, 20) =5.71, p = .005). Week four demonstrated marginally significant differences (F (3, 20) = 2.65, p= .076). Fluctuations in weight during week five and six also differed significantly between groups (F (3, 20) = 21.29, p= .001) and (F (3, 20) =19.46, p= .001), respectively. ANOVA results demonstrated a main effect of group membership on overall mean increase in weight over the first six weeks of treatment (F (3, 20) = 3.19, p= .046).

Tukey's HSD post-hoc analysis indicated that the Control and the EEs/Exercise group differed significantly in weight gain beginning in the 3rd week of treatment (p = .003). During week four the Stark environment group showed the greatest separation from the EEs/Exercised group (p=.048). Weeks five and six resulted in the EEs/Exercise group displaying significant variation from all other groups (p< .001). Finally, the mean increase in weight per week over the first six weeks showed the greatest difference between the Control group and the EEs/Exercised group (p= .035). None of the variation between the other experimental groups approached significance. Table IV illustrates each groups mean weight gained or lost per week.

Tests of association revealed that experimental group membership was significantly correlated with the overall mean increase in weight per week at the end of

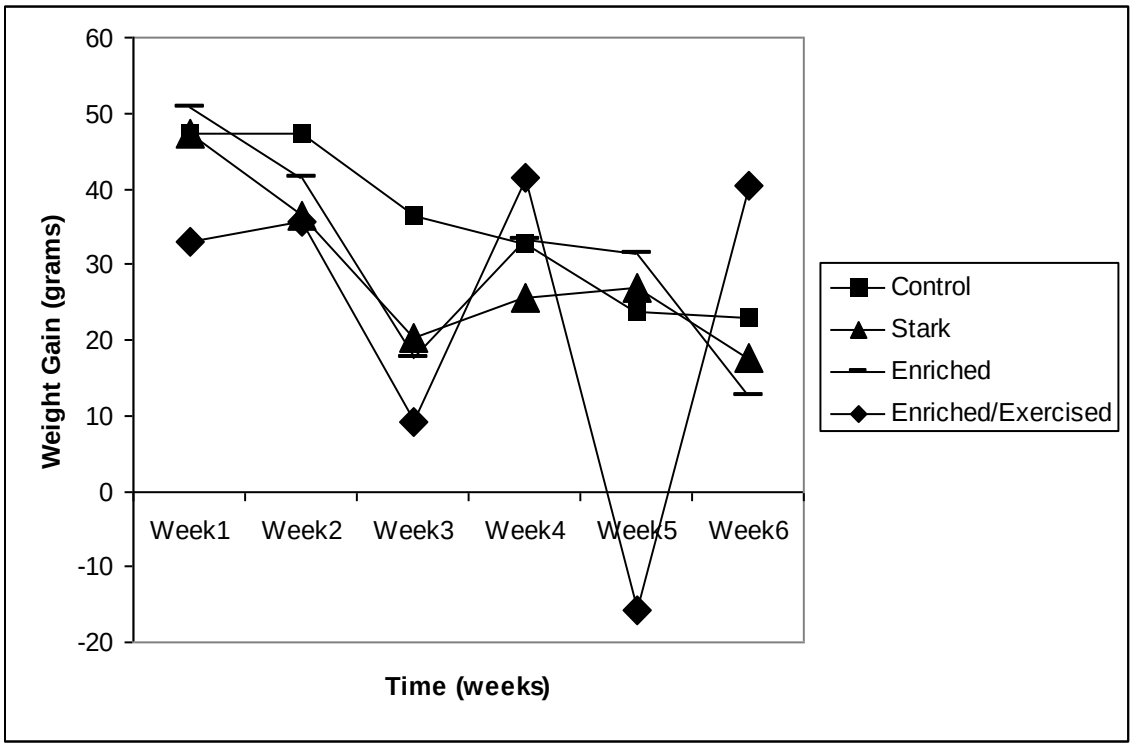

Figure 3. Plotted weight gain per week. Each experimental group's mean weight (g) gained per week for the first six weeks of CMS.

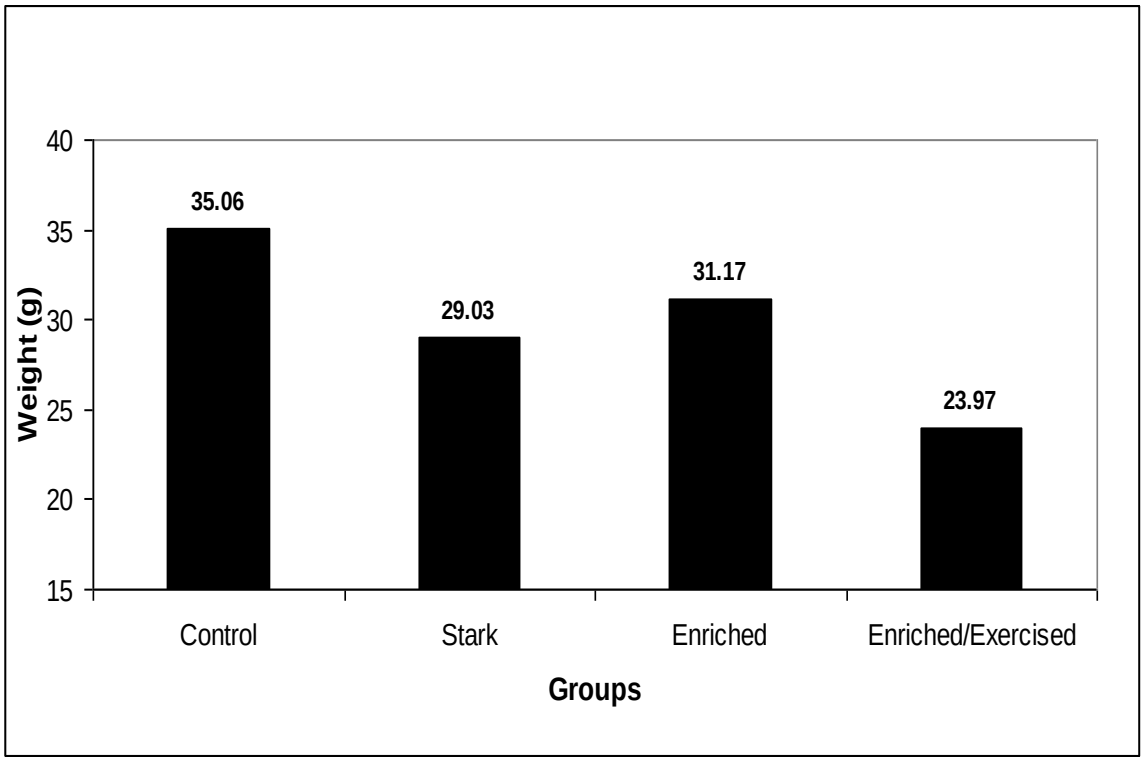

Figure 4. Mean overall weekly weight gain. Mean growth rate (g) per week for each experimental group over the entire six weeks.

Table IV.

Weight fluctuations per week. Data are represented as mean and ± standard error of the mean and were obtained weekly from all rodents included in the experiment. The number of animals in each experimental group was n=6 with N= 24.

AGE	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Overall
Control	47.3 ± 4.8	47.2 ± 4.1	36.5 ± 2.3	32.7 ± 2.7	23.7 ± 3.6	23.0 ± 3.4	34.4 ± 2.8
Stark Environment	47.3 ± 8.6	36.3 ± 4.9	20.3 ± 6.2	25.7 ± 5.3	25.3 ± 3.2	17.5 ± 3.7	28.3 ± 2.9
Enrich. Environment	50.7 ± 7.7	41.5 ± 3.0	17.7 ± 5.3	33.2 ± 4.4	31.3 ± 4.6	12.7 ± 1.4	31.2 ± 1.5
Enriched/Exercise	33.0 ± 3.2	35.7 ± 5.1	9.2 ± 4.5	41.3 ± 2.7	-15.7 ± 6.5	40.3 ± 1.7	24.0 ± 2.5

the first 6 weeks of treatment (r (24) = .470, p < .05). Increases in weight at the end of the 3rd week was also significantly associated with group membership (r (24) = .650, p < .05). The correlation between group and the increase in weight after the fourth week approached but failed to reach significance, while weeks five and six were both significantly associated with group membership (r (24) = .588, p < .05, r (24) = .435, p < .05, respectively). There was a strong negative association between the increase in weight at the end of week five and the increase in weight at the end of the 6th week (r (24) = -.685, p <.05).

3.1.1 SUCROSE TEST

At the end of the 8th week of treatment, the sucrose test was given as a measure of anhedonia. The results can be seen in Figure 5. There were evident differences in the amount of 1.5% sucrose consumed over the three day testing period. However, an analysis of variance of the quantity of sucrose solution consumed per experimental group approached, but failed to reach the level of significance ($F(3, 20) = 2.32$, $p = .106$, $\eta^2 = .258$). The quantity of sucrose consumption was not strongly associated with any other variable or measurement.

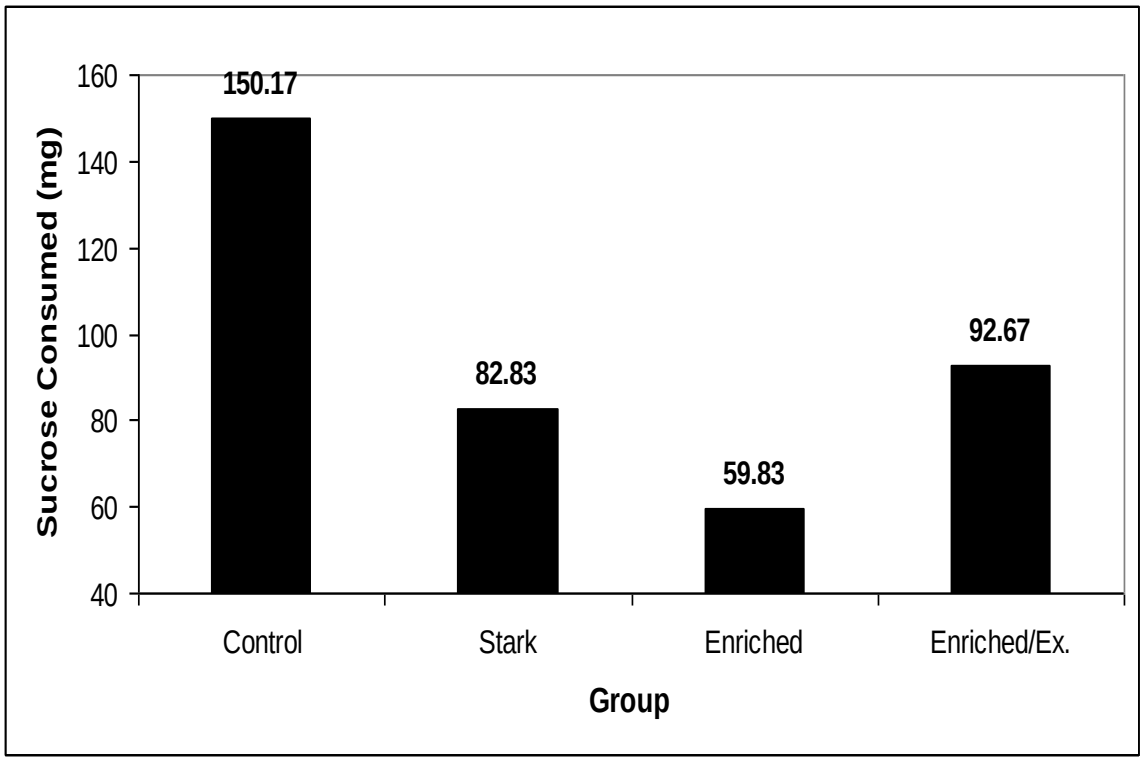

Figure 5. Sucrose test results. Mean volume of 1.5 % sucrose solution consumed per group over the three day testing period.

3.1.2 MORRIS WATER MAZE

At the end of the ten-week period of the CMS treatment each rodent underwent 10 trials of Morris water maze testing followed by an individual trial probe test. Performance in the water maze over the 10 trials was measured based on the distance swam per trial (path length) (see Figure 6). The mean path lengths (see Figure 7) for the individual groups did not reach significance ($F(3, 20) = 1.53$, $p = .24$). Path length significantly decreased between the first and last trial ($F(1, 9) = 11.31$, $p = .001$). Trial 3 revealed the largest variability in distances swam, an ANOVA identified a difference of marginal significance ($F(3, 20) = 2.96$, $p = .057$). No other trial displayed such a trend.

Closely associated to path length was the latency to locate the platform for each of the trials (see Figure 8). Resembling the path length data, a repeated measures ANOVA revealed that the overall mean latency for all groups to reach the platform decreased significantly between the first and last experimental trials ($F(1, 9) = 11.18$, $p = .001$). Again, mirroring the path length data, both trial 1 ($F(1, 9) = 73.24$, $p = .000$) and trial 2 ($F(1, 9) = 15.30$, $p = .001$) latencies were significantly longer in duration than the final eight trials. The differences in the experimental groups mean latency for the entirety of the experiment (see Figure 9) proved to be marginally significant ($F(3, 20) = 2.44$, $p = .094$, $\eta^2 = .268$). While there was discernible diversity in the latency between the groups over the 10 trials, the variation was not significant for any of the trials. Identical to the path length data, Trial 3 displayed the greatest variation between the groups ($F(3, 20) = 2.24$, $p = .115$). There were five blocks of trials during the MWM testing. Each block consisted of two identical trials. Analysis of the mean latency for each block indicated that the largest variation in performance took place during block 4, however the differences did

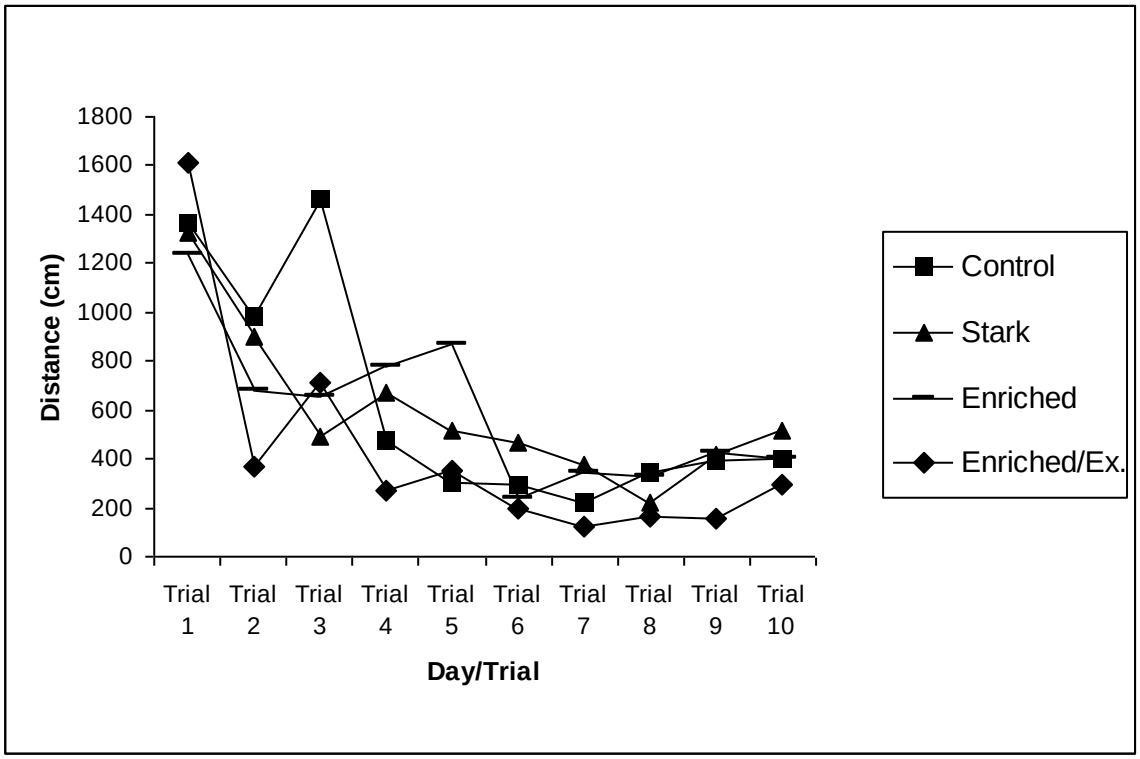

Figure 6. Plotted distance swam per trial. Each group's mean distance (cm) swam per Morris water maze trial.

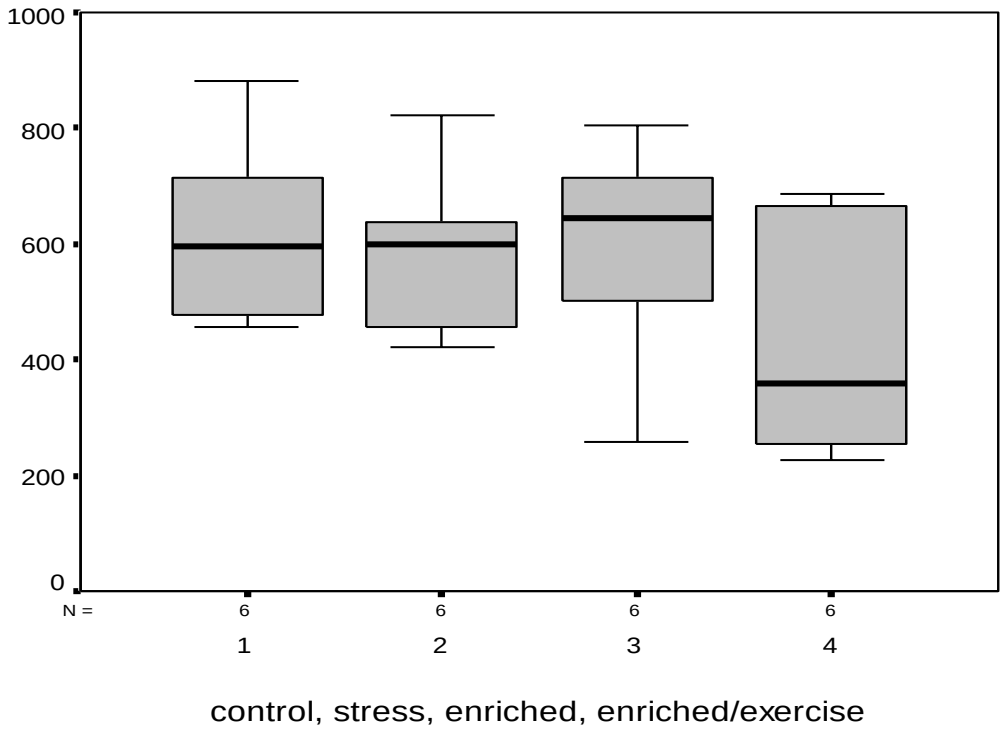

Figure 7. Mean path length. The mean distance swam for each group over the entire Morris water maze testing period.

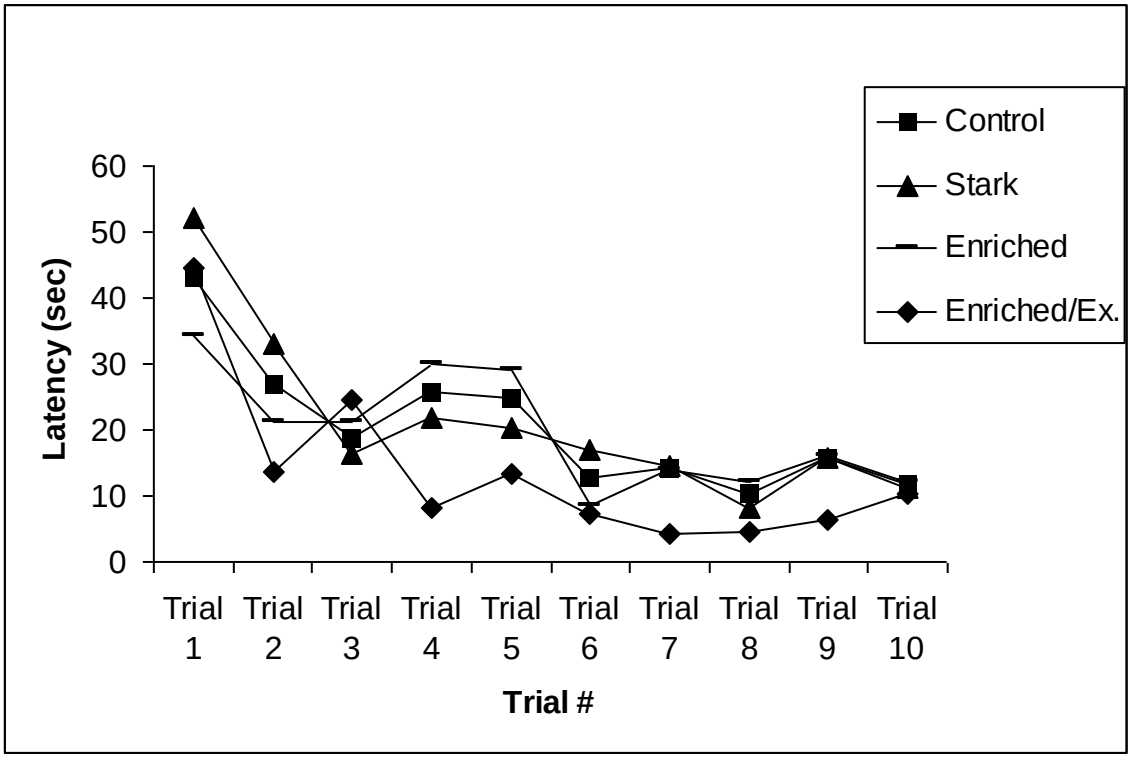

Figure 8. Plotted latency to locate platform. The mean latency to locate the platform for each group over the ten trials of Morris water maze testing.

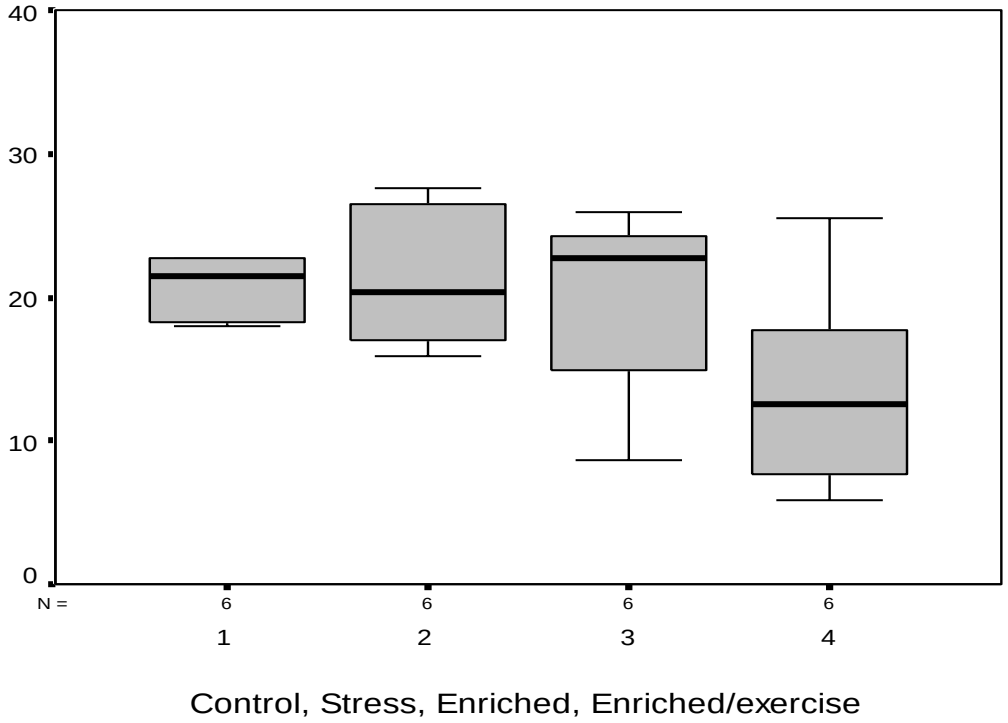

Figure 9. Mean MWM latency. The mean amount of time required for each group to locate the platform over the entire testing period.

not reach significance (F (3, 20) = 1.88, p= .166). The mean latency for trial 2 was found to be highly correlated with group membership, and the relationship proved to marginally significant (r (24) = -.398, p = .054). On the other hand, the association between the mean overall latency and group membership did reach significance (r (24) = -.469, $p < .05$).

Finally, the percentage of the trial latencies spent swimming in the platform quadrant was recorded for each trial (see Figure 10). An examination of each experimental groups mean percentage of time expended in the platform quadrant revealed a significant difference during trial 2 (F (3, 20) = 3.80, p = .026). A post hoc analysis revealed notable differences among the Control group and the Enrichment group (p = .046). Significant differences were also observed between the Enriched and the Enriched/exercise groups (p = .036). All other trials including the overall mean failed to approach significance (see Figure 11).

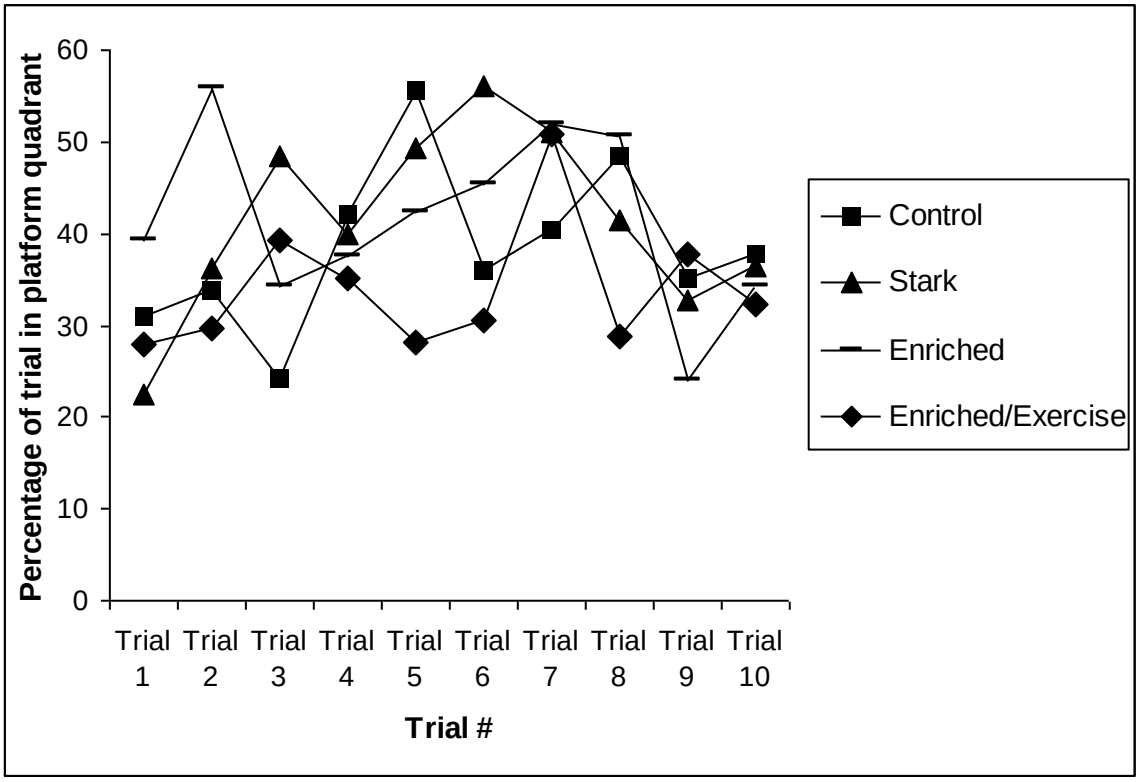

Figure 10. Plotted percentage of time in platform quadrant. The mean percentages of the trial latency spent in the platform quadrant.

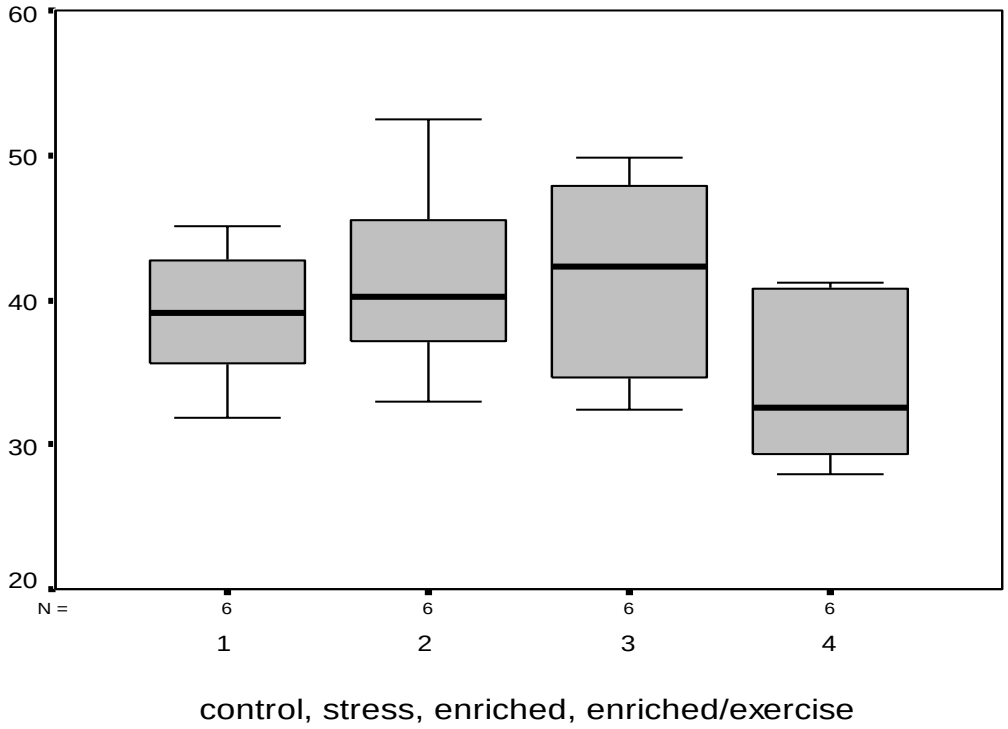

Figure 11. Mean percentage in platform quadrant. The overall mean percentage of the water maze trial latencies spent in the platform quadrant.

3.1.3 PROBE TRIAL

After completion of the 10 trials, the rodents were tested in a final probe trial in which the platform was removed. Performance on this trial was based on measurements of each rodent's mean proximity to the original platform location (see Figure 12), and also the number of entries made into the platform zone (see Figure 13). Analysis of the probe trial data revealed no significant differences between the experimental groups based on the number of entries into the platform zone $F(3,20) = .649$, $p = .593$) and also on the mean proximity to the original platform location $F(3, 20) = .698$, $p = .564$). Probe trial proximity and the number of entries into the platform area during the probe trial exhibited a significantly negative association ($r(24) = -.720$, $p < .05$). Interestingly, the increase in weight at the end of the first week of treatment was highly correlated with the rodents' proximity to the original platform location during the probe trial ($r(24) = .510$, $p < .05$), as well as the rodents' number of entries into the platform area ($r(24) = -.567$, $p < .05$).

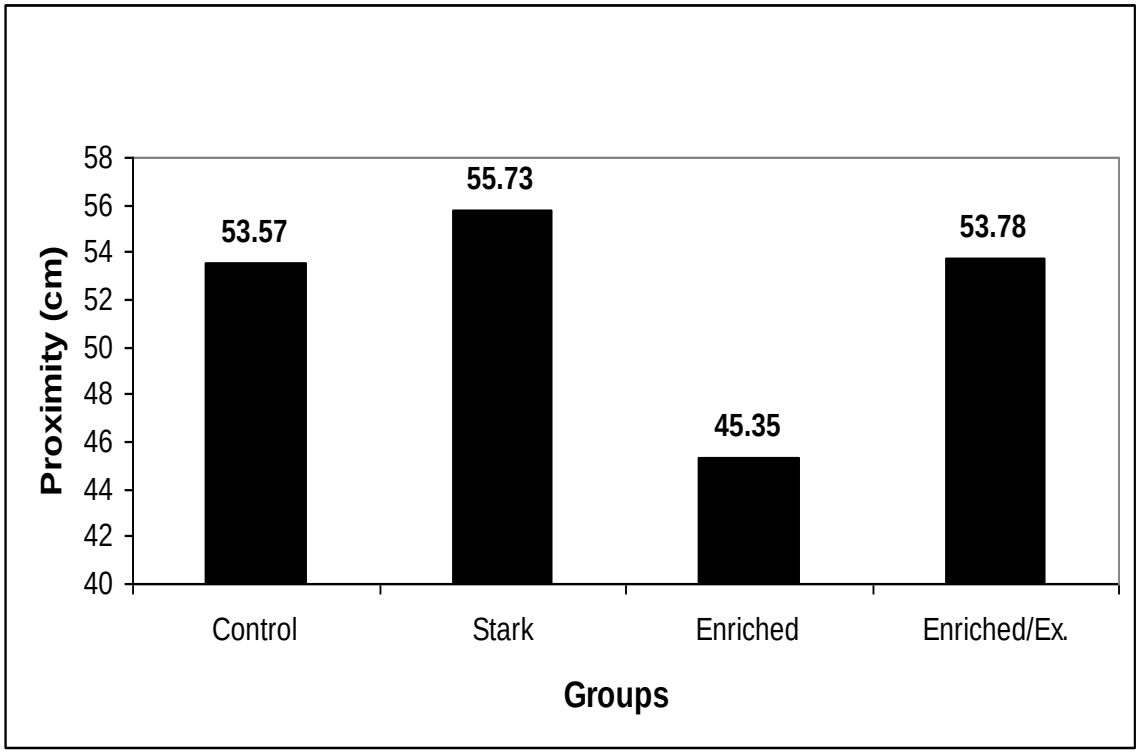

Figure 12. Probe trial proximity. The mean proximity (cm) for each group to the original platform location during the probe trial.

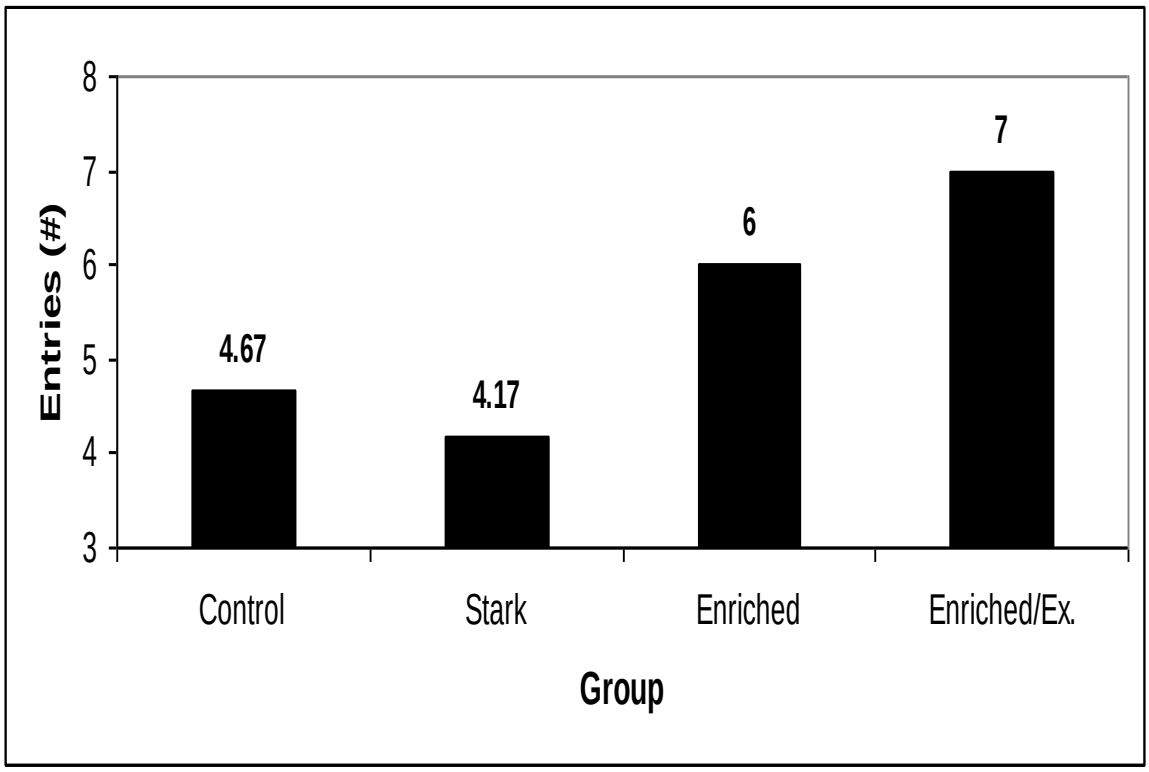

Figure 13. Probe trial zone entries. The mean number of entries into the platform zone per group during the probe trial.

3.1.4 NOVEL OBJECT PLACEMENT TEST

Each experimental group's mean difference in the percentage of time exploring toy 1 before and after it had been moved, was measured. (see Figure 14) Their percentage difference in their mean proximity to the toy in trial A and after it was moved in trial B was also recorded. (see Figure 15). No significant differences were observed between the groups based on their percentage increases in time spent exploring the moved object in trial 2 of the NOPT ($F(3, 20) = .938$, $p = .441$). There was also no significant differences based on each group's mean percent decrease in proximity to the moved toy $F(3, 20) = .239$, $p = .868$).

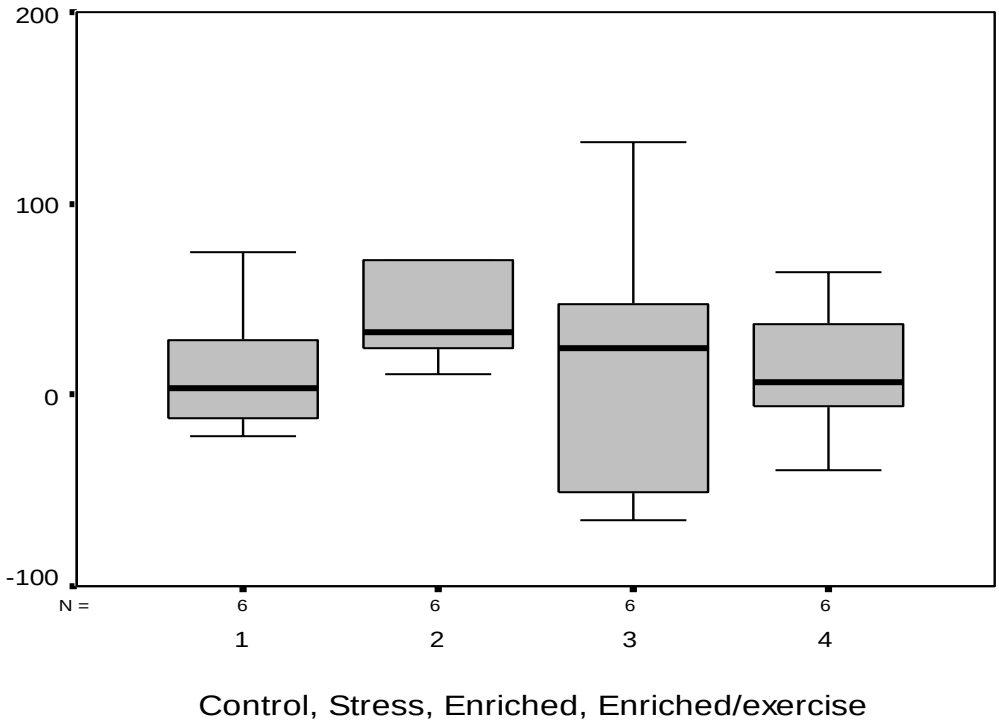

Figure 14. Percentage increase in time spent exploring moved toy. Displayed is each group's mean percentage increase in the amount of time spent exploring the toy after it had been moved..

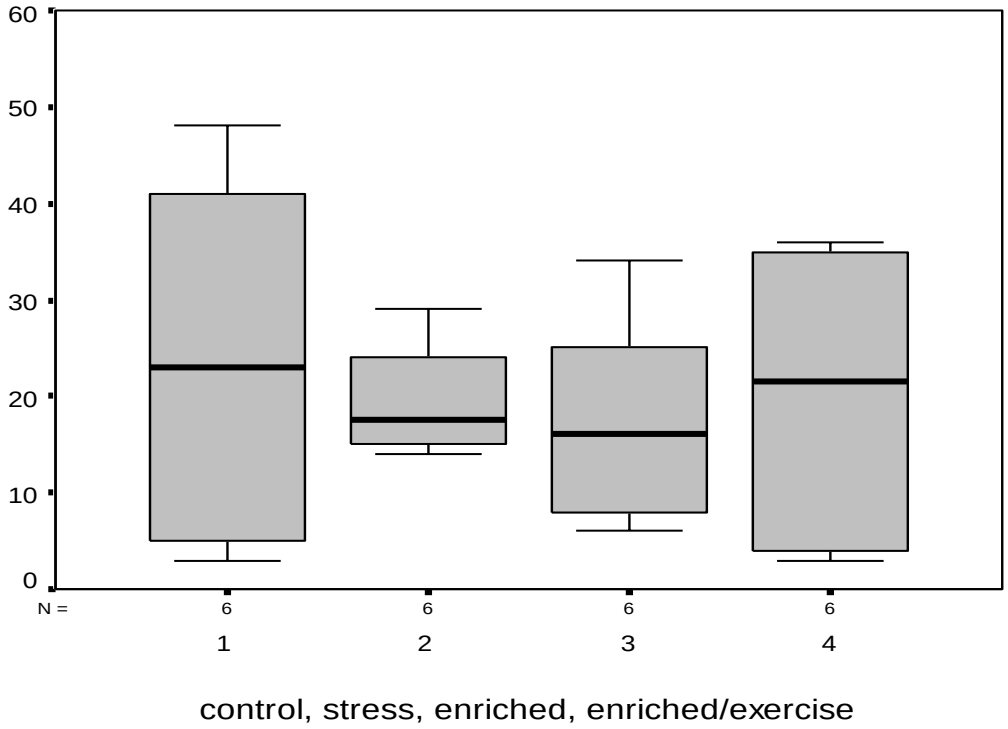

Figure 15. Percentage differences in the proximity to toy before and after movement. Displayed are the mean differences in proximity to the moved toy in trial 2 of the NORT and the proximity to the non-moved toy.

3.1.5 RUNNING WHEEL

Based on the data gathered by the PC from the running wheel cages, it was concluded that all of the rodents in the EEs/exercise group did run throughout their exposure to CMS. There were apparent differences in the volume of wheel running performed by the individual rodents in the EEs/exercise group (see Figure 16). However, analyses concluded that the distance ran did not influence the rodents performance on any aspect of the behavioral tests. The number of revolutions was associated only with the mean weight gained per week ($r(6) = .918, < .05$).

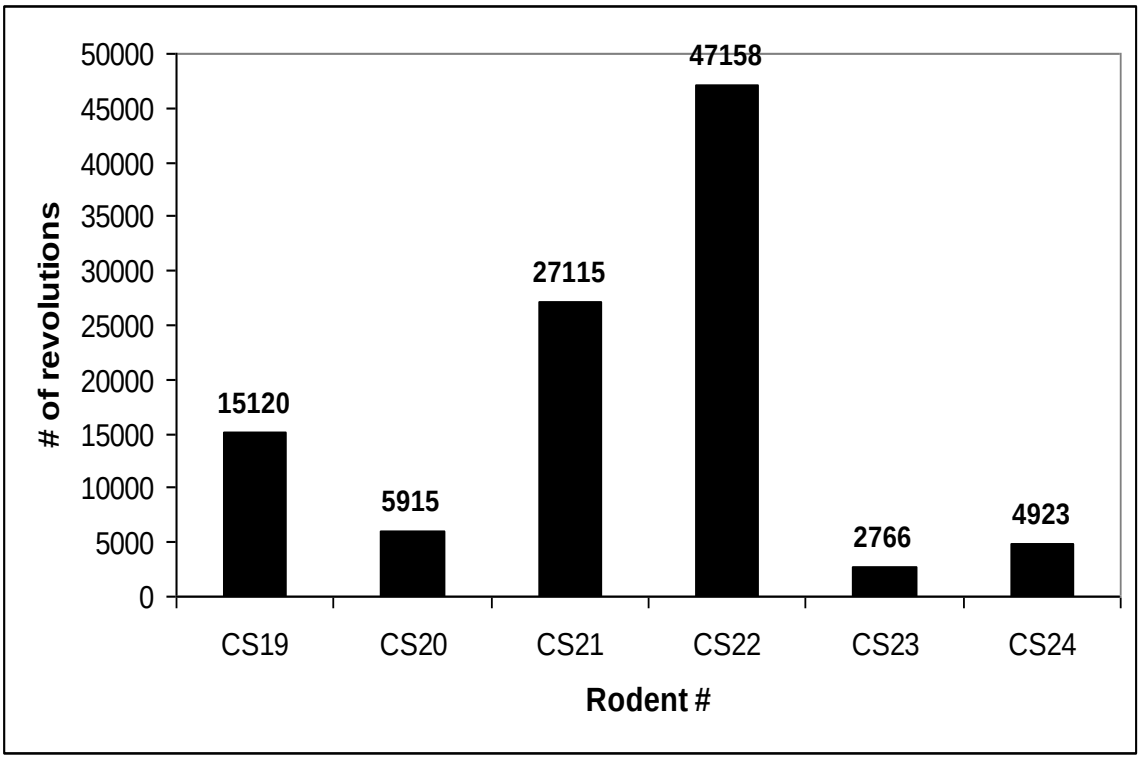

Figure 16. Number of revolutions ran. This graph represents the total number of wheel revolutions recorded from each rodent's cage over the last two weeks of CMS treatment.

CHAPTER IV

DISCUSSION

The purpose of this study was to further isolate the hippocampus as a structure involved in the formation and prevention of depression and depressive like symptoms. It was proposed that behaviors that result in greater hippocampal stimulation would be more effective in preventing the onset of these depressive symptoms.

- Weight loss
- Anhedonia
- Spatial memory impairment

The experimental treatments used in this study were chosen based on their known efficacy in enhancing hippocampal cell growth. Concurrent exposure to these treatments during the 10-week CMS period allowed us to identify the most effective form of prevention of specific depressive symptoms during times of prolonged stress. Our results point to a potential additive effect of environmental enrichment and exercise in combating the behavioral symptoms of depression.

Weight

The weekly increases in weight served as the primary indicator of the interaction between persistent stress exposure and the designated experimental treatment of the individual groups. Stress in rodents is associated with reductions in weight gained per week (Wright & Condrade, 2008). Therefore, the lower the weight, the more sensitive to stress that experimental group is believed to be. While the experimental groups' mean increases in weight were consistent with the expected trend the differences were too small to be definitive. Also, the weights from the EEs/exercise group were in conflict with the intended point of this measure. Lower weights in this group were more associated with higher amounts of running as opposed to more severe depressive symptoms. Based on each group's environment, lower weights should have been characteristic of those experiencing lower levels of stimulation.

The results showed a main effect for weight based on group membership however, the major differences in weight were observed between the Control and the EEs/exercise groups only. Although not significant, early differences *were* observed between the Control and Stark groups weight gain which would point to early variations in their responses to the environment, since the sole difference between these two groups was the Stark group's exposure to CMS. Other studies have observed such early and persistent fluctuations in weight gain as a result of the CMS treatment (Forbes, Stewart, Matthews & Reid,[1] 1996).

While the validity of this measure is not in question, the present study's utilization

[1] The Enriched/Exercise groups weights would naturally be significantly lower as a result of the greater number of calories burned, regardless of their sensitivity to the CMS treatment.

of it made it difficult to identify differences in weight based on the intensity of the symptoms as opposed to the level of activity demonstrated by the experimental groups. Weight would be a more suitable index if the experimental design provided all groups with access to exercise, or none at all.

Anhedonia

Decreased sensitivity to pleasurable experiences or anhedonia is a known symptom of depression. Sucrose consumption was used as an indication of depressive symptoms at the end of each rodent's 8th week of CMS. While there were differences between the amounts consumed between groups, they approached but did not reach a level of significance. The EEs totals conflicted with our final hypothesis. It was expected that rodents receiving more stimulation would consume more sucrose, and while this was observed slightly in the EEs/exercise group none of the differences proved to be significant. However, group membership did account for 25% of the variance in overall consumption.

Sucrose solution consumption can be attributed to changes in physiological sensitivity to the taste, as opposed to reductions in overall drinking, since the intake of water is unaffected by CMS (Willner, 1997). Also, anti-depressant treatment has been shown to increase consumption in stressed animals but not controls, demonstrating a chemically induced restoration of sensitivity in anhedonic rodents (Muscat, Papp & Willner, 1992). Therefore, the variations in the amounts consumed cannot be explained by factors relating to thirst, but are instead caused by physiological changes more closely believed to be related to pleasure avoidance (Forbes, Stewart, Matthews & Reid, 1996).

It is possible that the rodents in the EEs group were preoccupied with the environmental and dietary enrichment and therefore were less likely to spend as much time drinking the solution; however, the mean volume consumed by the EEs/Exercise group would suggest otherwise, since both groups received similar types of stimulation. It is certainly viable that the EEs group did indeed experience more severe depressive symptoms than the Stark group as a result of the CMS treatment. However, resilience to the onset of anhedonic behavior after long-term exposure to chronic unpredictable stress has been observed in some studies (Gouirand and Matuszewich, 2005; Henningsen, at. al., 2009). Indeed, the cognitive deficits typical of depression are still observed in stressed rodents who are found to be less susceptible to anhedonic-like behavior. Therefore, the results of this test are difficult to interpret.

Water Maze

Results from the MWM testing were less ambiguous. Since the outcome of the analyses of path length and latency were so highly similar, only latency and the percentage of time spent swimming in the platform quadrant will be discussed. It was predicted that the rodents receiving enrichment during their treatment would learn the location of the platform earlier and reach it more efficiently than the Stark group, thereby demonstrating superior spatial memories. In terms of the mean latency for each group over the 10 testing trials, the differences between the groups attained marginal significance. The EEs/Exercise group performed at the highest level in terms of their ability to locate and retain the location of the platform, which was consistent with our 2nd hypothesis. Their mean time required to reach the platform was 36% lower than the mean

time of the rodents in the Stark environment group. Curiously, the EEs and the Stark groups performed similarly. Such a small disparity in water maze performance between the EE and Stark groups seemed to support the results of the sucrose consumption test, and provide added evidence for their comparable depressive states.

Group membership was strongly associated with mean latency over the course of the MWM testing indicating identifiable differences in performance. The largest differences observed in trial latency occurred during the initial trials. Early differences would be expected if the CMS had affected each group differently depending on their designated treatments. Rodents should locate and retain the position of the platform at a different rate. In fact, trial 2 and 3 showed the greatest variation between groups in latency as well as the percentage of time spent in the platform quadrant, pointing to the expected difference in the rate of spatial memory storage among the groups. Normalization of each group's latencies beginning in trial 1 and continuing on to trial 10 showed no significant differences in the percentage reduction in time for any successive trial. This may have been caused by the variations in each testing groups starting distance to the platform for each block of tests, which would lead to apparent fluctuations in the percentage decrease. In other words, trial 2 for the first testing group may have had a large decrease in latency due to closer starting proximity to the platform, while trial 9 may have been further away but the latencies may have been similar indicating minimal differences in the percent reduction, even though learning may have continued to take place throughout testing. Still, all of the rodents did manage to learn the exact location of the platform before the end of the testing period.

While the EEs/Exercise group demonstrated a perceptible advantage in the storage

and retrieval of the platform location, they spent the smallest percentage of their trials in the platform quadrant. This group spent 7% less time in this quadrant than the Stark group and the EEs group who performed almost identically on this measure. Interpretation of this data is difficult since it could be assumed that greater time spent in the platform quadrant is indicative of greater awareness of the platform location. However, the researchers suggest an alternative explanation for this discrepancy. It is more likely that additional time spent in the platform quadrant represents greater uncertainty as to the platform's exact location. Rodents spending a greater percentage of time swimming in the platform quadrant would naturally have to swim longer in this quadrant without locating the platform. Therefore, these results are most likely a measure of the precision of the spatial memory ability of each group. Based on this assumption, the EEs/Exercise groups displayed greater accuracy and efficiency in locating the platform than all other groups. The results from the MWM further support the strong similarities in spatial memory performance between the Enriched and Stark groups, indicating no advantage in the level of spatial memory ability maintained through consistent enrichment.

Probe Trial

The purpose of the probe trial was to measure each group's retention of the precise location of the platform by tracking each rodent's time spent swimming on or around the area where the platform had originally been placed. Like the MWM, it was predicted that higher levels of enrichment would result in more well preserved spatial memory. The probe trial measurements were somewhat consistent with the MWM findings. In support

of our interpretation of the results from the MWM, the EEs/Exercise group on average crossed into the original platform area more than any other group providing further evidence for a more precise spatial memory in this group. The EEs group performed similarly, while the Stark group demonstrated less specificity in their search for the platform. While the results indicate a perceivable divergence in performance, the group variations in entries didn't reach significance.

The EEs group swam in closest proximity to the platform location during the probe trial, while the EEs/Exercise groups swam on average 16% further away from the spot of the original platform. The Stark groups mean proximity was slightly higher than the EEs/exercise group's but the differences were minor. In terms of the percentage of time spent in the platform quadrant the results were similar to those obtained during the MWM testing. The EEs group spent a markedly longer percentage of time searching in the platform's quadrant than the other groups. Mirroring the MWM results, the EEs/Exercise group occupied the platform quadrant the least during the one-minute trial. Again, this is not a direct indication that the EEs/Exercise group had impaired spatial memory, although that seems to be a reasonable assumption. However, based on their low mean latency to find the platform this is difficult to accept. Instead, it may be more probable that the EEs/Exercise group became aware of the absence of a platform faster than the other groups. As a result, these rodents would have moved into other quadrants to explore further rather than spend more time in an area that no longer provided safety. The greater number of platform zone entries by the EEs/Exercise group also supports our reasoning.

Similar studies have also failed to observe significant differences in time

spent in the platform quadrant (Gouirand and Matuszewich, 2005). In this study, the EEs group consistently spent more time searching for the platform in areas closer to its original location not only in the MWM but also during the probe trial. Therefore, it was concluded that while the memory of the general position of the platform was stronger in the EEs group than the Stark group, the accuracy of both groups' spatial memory was inferior to those who had access to exercise. The Enriched group's lower proximity to the original platform location in both tests along with the greater amount of time searching in its quadrant supports the possibility of an inaccurate but persistent general awareness.

Novel Object Placement Test

The results of the NOPT were somewhat contradictory to the hypothesis and appeared incompatible with the other tests. It was predicted that greater time spent exploring the moved toy in trial B was indicative of more well preserved spatial memory. Therefore, the researchers expecte that the groups receiving enrichment would demonstrate a clear bias towards the moved objects. The means of the groups on both measures of this test were too close to identify any significant differences. In terms of the difference in time spent exploring the two toys, the EEs/Exercise and Stark groups performances were indistinguishable. The Control group showed the smallest preference for the moved toy, which was also not anticipated. The difference in proximity to each toy in the second trial conflicted with the previous results if proximity in this test is an indication of general curiosity. The Stark group spent the second trial 59% closer to the moved toy than did the EEs/exercise group. The difference in proximity may not have been a reliable indicator of preference in the behavioral tests utilized in this study. Other

studies have also failed to find significant differences in exploration time between stressed and non-stressed rodents (Li, et al., 2008). No identifiable trends in performance were found and this is could be due to the small number of rodents tested as well as the relative insensitivity of the test measures. The design of this test assumed that rodents would be capable of identifying whether an object moved or did not move based solely on the stationary object's consistent position in front of a spatial cue inside the pool. In fact, both objects may be considered to have moved if left/right orientation was the subjects' primary means of determining location. More specifically, if the object that was originally on the rodent's left side in trial A is now located to its right in trial B, then the same would apply for the non-moved toy. Both objects essentially change their orientation relative to the rodent. The only identifiable consistency in the positioning of the non-moved toy was its maintained proximity to a specific visual cue inside the pool. Therefore, this test demanded a level of spatial memory acuity that its design was perhaps too insensitive to measure.

Interesting associations were observed between the time spent exploring the toys and other measures in the test that should be noted. For example the time spent exploring both toys during the first trial was negatively associated with the rodents' mean weight gain per week indicating greater exploratory behavior in the lighter rodents. Also, an interesting negative correlation was discovered between the latency in trial 3 and the difference in exploration time between the two toys. In other words the lower the rodent's latency in this trial, the greater the rodent's bias towards the object in the novel position. As noted before, trial 3 experienced more variation in latency between the groups than any other trial. It would seem that performance in this trial was more predictive of overall

spatial memory ability than any of the others. Indeed, the earlier phases of testing were the strongest indicators of any variation in overall spatial memory ability.

Conclusion

Stress or the exposure to sufficient levels of GCs results in observable damage to the hippocampus (Stein-Behrens, et al. 1994). Reductions in the firing rate of hippocampal neurons have also been observed (Duman, Malberg, Nakagawa and D'Sa, 2000). The negative response of these cells as a result of exposure to GCs is linked to significant reductions in overall activity as well as the eventual atrophy of the neurons that is common among depressed patients. These lower levels of neurological excitement and the eventual reductions in the volume of the hippocampus are associated with identifiable impairments in memory performance (Kirschbaum, et. al., 1996, Ohl, et al., 1999). This study did not measure the severity of the depressive symptoms on a cellular level but instead observed the physical and behavioral effects induced by weeks of uninterrupted CMS exposure. Performance on the behavioral tests was intended to be an indication of some level of hippocampal impairment.

While the stress provided for the groups remained identical throughout the 10 weeks of CMS treatment, the rodents' sensitivity to the stress was manipulated through the introduction of new and unfamiliar objects as well as the ability to exercise. Exercise actually increases GC levels in the blood but does not have a harmful impact on the functioning of the hippocampus (Christie et al., 2008; Gomez-Pinilla, Dao & So, 1997). Exercise is believed to be a modulator of the HPA axis. Perhaps it is the steady exposure to low levels of GCs that allows for the habituation and eventual increased resistance to

stress. Hattori et al. (2007) and Markham (2004) propose the same explanation for the beneficial effects of enrichment in reducing the severity of depressive symptoms. Frequent exposure to new and unfamiliar objects can be classified as a series of mild stressors. Several mild stressors over the course of a ten week period would certainly have an effect on subsequent HPA activity. Recurrent exposure to mild stressors would eventually desensitize the rodent to the stress of novelty allowing it to engage in more exploratory behavior in unfamiliar surroundings.

Several studies have observed increases in growth factor concentration in the hippocampus as a result of heightened levels of physical activity (Gomez-Pinilla, Dao and So, 1997; Van Praag, 2009). Higher levels of BDNF are typically accompanied by increases in hippocampal cell proliferation (Bjornebekk, Mathe and Brene, 2005). Therefore, it was concluded that hippocampal maintenance in any form would increase one's resistance to developing depressive symptoms. Hippocampal stimulation is most certainly not isolated to exercise and EE only. There are undoubtedly additional methods of promoting higher levels of activity in the hippocampus however exercise and EE are known environmental and behavioral means of achieving this result.

The present study intended to focus on providing hippocampal stimulation as a means of potentially attenuating the behavioral and cognitive symptoms of depression. Treatments were chosen based on their positive neurogenic properties, specifically in the hippocampus. The presence of these treatments during a prolonged period of CMS revealed the potential importance of consistent hippocampal maintenance when faced with extended periods of chronic stress. This study also takes a step toward examining the interactive effects of multiple treatments on the preservation of cognitive ability, as

opposed to only one.

The overwhelming majority of related studies have focused on the treatment of previously induced depressive symptoms as opposed to possible forms of prevention or attenuation. The current study examined solely the protective ability of these practices. Earlier studies have also failed to examine the potential additive benefits of combined treatments. It is unclear whether exercise and environmental enrichment share potential additive benefits when practiced in combination. Yet it *is* clear that EE and exercise provided a greater amount of resistance against the development of depressive symptoms than just EE alone, which did not appear to afford any noticeable advantage in the prevention of these symptoms. Increased cell proliferation and growth factor concentration were likely factors that influenced this result, although it was not our aim to affirm that idea. Evidence from previous studies supports this conclusion. The lack of statistical power and sensitivity in this study prevented the identification of significant variation between groups in several measures, although clear differences in spatial memory ability and responsiveness to stress were apparent.

While the results from this study point to an advantage in performance for those rodents receiving both EEs and exercise, there was certainly room to improve the design of this study. Water maze testing would be the primary measure of spatial memory, as opposed to two separate tests. Such high frequency testing during each rodent's last week of experimentation may have interfered with the results of the novel object placement test. Regardless of whether the outcome of the novel object placement test was valid, it added little to the overall results of the study and therefore would be omitted in future studies. Most importantly, if done again this study would examine the benefits of exercise

in isolation in order to verify that there was indeed an additive effect of combined exercise and EE. The design of the present study did not allow for the measurement of exercise's preventative capacity when provided in isolation.

Future research should focus not only on identifying the potential interactions of these behavioral treatments, but also on the identification of other treatments that are capable of eliciting similar levels of stimulation in the hippocampus. There may also be a discrepancy in the effectiveness of each treatment based on the time of their administration. In other words, enriched environments may be more successful at treating previously developed depressive symptoms, while exercise may be the best proactive method of treatment. Providing long-term exposure to exercise and enrichment prior to the administration of stress may be another possible means of examining the protective efficacy of these treatments. Comparing the rate at which previously enriched rodents develop depressive symptoms after their introduction to stress with that of rodents with no prior enrichment might further illustrate the potential stress-attenuating influence of these treatments. Future variations of this study may also help to identify the actual speed at which these protective physiological modifications are activated. By examining the spatial memory ability of chronically stressed rodents after a single period of exercise, it might be possible to identify immediate improvements, which would indicate a more instantaneous effect of these treatments on hippocampal function.

The results of this study suggest that depression and chronic stress can have damaging effects on both the hippocampus and its influence on behavior while exposure to exercise and environmental enrichment may lead to observable improvements in hippocampal activity and performance. Future research should aim to provide a better

understanding of the mechanisms by which these and other treatments improve mood and cognitive performance. Improved awareness of the kinds of experiences and practices that stimulate an enhanced defense against these symptoms will provide evidence in support of the types of lifestyles that may be most resistant to the cognitive and emotional consequences of chronic stress. Finding the answers to these questions could potentially lead to an optimization of the effectiveness of behavioral treatments in treating and preventing the symptoms of chronic stress and depression.

From an evolutionary standpoint, these results shed some light on the sort of behaviors that may have been the most auspicious in terms of increasing the odds of human survival. Perhaps both were essential ingredients for survival throughout earlier more life-threatening times in our species history. An existence consisting of regular physical activity, with exposure to novel environments would likely increase the chances of locating previously undiscovered sources of sustenance and habitation. Frequent exercise would assist not only in the tracking of game, but in the avoidance of predators. It is assumed by these researchers that for the majority of human existence, our way of life consisted of regular amounts of both environmental enrichment, and exercise. It is logical to imagine that over several generations of such a high-risk lifestyle, the behaviors and experiences that were consistent with improving the likelihood of survival would eventually become required for the healthy functioning of our bodies *and* minds. The human brain may have evolved a physiological need for the type of stimulation this lifestyle provides. If the human hippocampus has in fact developed based on the fundamentals of primitive life, then depression should be as widespread as it is in today's comparatively relaxed existence. An understanding of human development throughout

our history, as well as a familiarity with the basic ingredients for survival during these primitive times would aid in the detection of other potentially stress minimizing behaviors.

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