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The Behavioral Effects of Environmental Enrichment in Rats

by

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES

FIGURES

SECTION I - INTRODUCTION

Overview

Physical and social components of an environment can influence the behavior and biology of organisms. Environmental enrichment can enhance learning, memory, and improve information processing (Smith, 1972; Gardner et al., 1975; Daniel et al., 1999; Van Praag et al., 1999; Varty et al., 2000; Woodcock & Richardson, 2000). Studies of environmental enrichment have focused on changes in learning and memory, as well as, changes in neuroanatomy or cytoarchitecture of the brain (Hebb, 1947; Rosenzweig, 1966; Rosenzweig, Bennet, & Diamond, 1972; Rosenzweig & Bennet, 1996; Greenough & Jurask, 1979; Hall, 1998; Joseph, 1999; Pham, Ickes, Albeck, Soderstrom, & Mohammed, 1999; Van Praag, Kempermann, & Gage, 1999; Passineau, Green, & Dietrich, 2001). The clinical relevance of environmental enrichment may extend beyond learning and memory changes because enrichment also may affect behaviors relevant to an organism's health (e.g., feeding, body weight, behavioral responses to stress).

The purpose of the present experiment was to determine if rearing rats in enriched environments alters subsequent behaviors and responses to an experimental stressor. The present experiment included two specific aims: (1) to examine effects of environmental enrichment on behaviors that index health, activity, and levels of stress, and (2) to determine whether environmental enrichment reduces effects of stress on the behaviors examined under Specific Aim 1. As background for the research, Section I reviews the literature on

environmental enrichment and stress, primarily relevant animal research. Section II presents the rationale for each independent and dependent variable. Section III presents the hypotheses, methods, and data analytic strategy, and results for the experiment. Section IV presents a discussion of the findings including implications, limitations, and future research directions. Section V presents relevant tables, figures, and references.

Enriched Environments

Historical Context of Enriched Environments

Charles Darwin (1875) reported that the brains of domestic rabbits were considerably smaller compared to the brains of wild rabbits. He argued that the reduced brain size of the domestic animals was a consequence of a deprived environment because domesticated animals did not exert their intellects, instincts, or senses as much as animals did in the wild. Despite the importance of Darwin's observation, empirical support for his interpretation did not appear in the literature until decades later.

Donald Hebb (1947) observed that laboratory rats that he had taken home for his children to play with exhibited superior performance on maze learning when compared to rats kept in the laboratory environment. Hebb concluded that nerve cells in the brains of the rats had changed in response to the enriched and varied experiences outside the laboratory. He hypothesized that the number of synaptic connections increased and that these structural changes resulted in functional (i.e., behavioral) modifications. Hebb believed that these changes reflected new learning. This particular report of Hebb that was remarkably

consistent with Darwin's (1875) observation still did not generate research for almost 20 more years.

Mark Rosenzweig (1966) introduced the classic paradigm for studying the impact of enriched environments on rats. Animals are housed in groups to provide opportunities for social interaction (i.e., social enrichment). Physical stimulation (i.e., physical enrichment) involves providing objects in the cages to allow tactile stimulation and physical activity (Rosenzweig & Bennett, 1996; Woodcock & Richardson, 2000). Most subsequent environmental enrichment studies (e.g., Mohammad et al., 1993; Pham et al., 1999) include social and physical enrichment components. Enriched environments are distinguished from non-enriched environments by the amount of stimulation and activity available in the environment. The standard non-enriched environment limits the physical and social enrichment by housing the animals individually without objects (Varty, Paulus, Braff, & Geyer, 2000). Commonly, across human and animal research, environmental enrichment refers to physical and social stimulation provided in the environment.

Effects of Enriched Environments

Enriched versus non-enriched housing environments have different biological and behavioral consequences. This section briefly reviews biological and behavioral consequences of environmental enrichment.

Biological Effects of Enrichment

Although human beings are born with 100 billion neurons surrounded by over one trillion glial cells that protect and nourish these neurons, the pattern of "wiring" necessary for communication between the cells is not yet stabilized (Joseph, 1999). For example, the number of synapses in one layer of the visual cortex increases from approximately 2,500 connections at birth to as many as 18,000 connections only six months later (Kliem et al., 1998). Environment may contribute to the exact wiring that occurs because differential environments alter brain cytoarchitechture (Mohammed et al., 1983; Rosenzweig, 1996; Diamond, 2001).

Animal experiments reveal that enriched experience evoke the same cascade of neurochemical events that cause plasticity alterations in the human brain (Rosenzweig & Bennett, 1996). Stimulating environmental conditions (i.e., enriched environments) significantly influence brain development and functioning including: increased size and weight of the cortex, increased neuron sizes and dendritic branching, increased synapse formation, and elevated protein levels (Rosenzweig, Bennett, & Diamond, 1972; Mohammed et al., 2002). Diamond (1991) reported that laboratory rats housed in enriched environments could have up to 25 percent more neurons in their brains when compared to non-enriched rats. Along with biological changes in animals, behavioral changes have been reported.

Behavioral Effects of Enrichment

In addition to the increased number of neurons in the brain, rats reared in an enriched environment exhibit more complex behaviors than rats reared in non-enriched environments (Mohammad et al., 1993; Pham et al., 1999; Kobayashi, Ohashi, Ando, 2002). Environmental enrichment can significantly improve the cognitive functioning of animals on behavioral tasks of attention, memory, and learning compared to animals reared in standard non-enriched environments. For example, early social isolation leads to an interruption of attentional processing in rats as measured by acoustic startle reflex (Robins, 1996). Also, rats deprived of social contact post-weaning (i.e., when social play normally develops) have impaired information processing as measured by pre-pulse inhibition (PPI) of the acoustic startle reflex. PPI is believed to index an innate sensory motor "gating" mechanism that underlies the organism's ability to select relevant stimuli from the environment while screening out irrelevant information (Swerdlow, Caine, Braff, & Geyer, 1992).

Superior learning and memory task performance by rats reared in enriched environments is well documented (Greenough & Juraska, 1979). Woodcock and Richardson (2000) reported superior information processing and working memory for rats raised in enriched environments compared to rats raised in non-enriched environments. Rats reared in enriched environments were better able to discriminate between a conditioning cage and a similar but distinct cage. The Morris water maze and the radial maze tasks are widely used measures of rodent learning and spatial memory. When compared to non-environmentally

enriched rats, the enriched rats perform significantly better in the Morris water maze task (Daniel, Roberts, & Dohanich, 1999; Williams, Luo, Ward, Redd, & Gibson, 2001) and the radial maze (Juraska, Einon, 1980; Henderson, & Muller, 1984). Enriched housing environments also result in more rapid decreases in locomotor activity in novel environments, indicating faster learning and adaptation to the new environment. Similarly, rats reared in enriched environments display quicker adaptation of the acoustic startle response (Swerdlow, Caine, Braff, & Geyer, 1992).

Other animal research has revealed that reduced sensory stimulation results in performance deficits in learning tasks and hyperemotionality, whereas enhanced stimulation leads to improved performance and a significant reduction of emotionality (Haywood & Tapp, 1966). Stereotypic behaviors in animals are commonly thought to represent anxiety or stress (Grindrod and Cleaver, 2001). Grindrod and Cleaver (2001) reported that incorporating novel toys and opportunities to work for food reduced captive seals' stereotypic circling behavior. Additionally, pigs reared in enriched environments exhibited more diverse behaviors than pigs reared in non-enriched environments (Wemelsfelder et al., 2000).

In sum, enriched environments, characterized by the presence of physical objects and the opportunity for social interaction, have shown robust positive consequences. In contrast, non-enriched environments disrupt cognition and behavior. Stimulating or enriched environments enhance healthy brain development and provide marked improvements in performance.

Stress

Historical Context of Stress

Stress is the process in which an organism responds to reduce the impact of internal or external events (i.e., stressors) that threaten or challenge the existence and well-being of that organism (Baum, Singer, & Baum, 1981; Baum, Grunberg, Singer, 1982; Baum, Gatchel, & Krantz, 1997). Walter Cannon (1935) suggested that organisms respond to events or challenges to an internal homeostasis with reactions that attempt to restore a balance within the body. He recognized that these various responses appeared to facilitate an organism's survival (i.e., the fight or-flight response). Cannon (1935) also indicated that illness results when an organism is chronically activated in maintaining homeostasis in response to an imbalance caused by environmental events. Hans Selye (1973) identified negative consequences of chronic stress, specifically that stress or chronic biological activation resulted in illness. According to Selye's (1973) General Adaptation Syndrome (GAS), stress is a non-specific response of the body to demands for adaptation, primarily involving the Hypothalamic-Pituitary-Adrenal (HPA) Axis. Specific events, positive or negative, activate the HPA Axis resulting in various biological responses. He believed that the manifestation of stress is a strictly autonomic biological response and that the long-term effects of the HPA activation resulted in disease and health impairment (Selye, 1973).

Several other investigators studying the stress processes recognized that life events and non-physical (psychosocial) challenges to an organism also result

in stress responses. John Mason (1974) presented evidence that different stressors resulted in different hormonal profiles and asserted that the non-specific responses to diverse stimuli were the result of the psychological experience of stress. Mason suggested that the individual's experience of stress depends on one's appraisal of a situation or stimulus, personality factors, situation or environmental influences, and an integrated multi-hormonal response (Mason, 1974). Rahe and Arthur (1978) attempted to quantify stress-inducing events to determine vulnerability to illness related to an individual's level of stress. Based on Tuke's (1884) proposition that dramatic life events evoke strong emotional responses and disease states, Rahe and Arthur (1978) attempted to account for environmental, sociological, psychological, and physiological characteristics suggesting that the bases for stress are psychosocial events imposed upon perceptual systems of cognitive functioning that are transformed into physiological events.

Richard Lazarus and colleagues emphasized the contribution of cognitive factors in the individual's response to a stressor. In their view, stress encompasses three processes: threat, appraisal, and coping (Lazarus, 1966; Lazarus & Folkman, 1990). Threat denotes a state in which a person anticipates harm. Appraisal specifies the process in which a person evaluates cues to assess future conditions. Coping processes allow an individual to reduce or eliminate the anticipated harm if a stimulus has been perceived as threatening. Individual cognitive styles use these processes to determine if a stimulus is perceived as stressful or not stressful. Additionally, perceived controllability and

predictability (i.e., cognitive control) over a source of stress also determines a person's response to stress (Glass & Singer, 1972; Grunberg & Singer, 1990). The history of stress research reviewed here suggests that biological, psychological, and environmental variables are relevant to stress responses. Therefore, biological, psychological, and environmental factors are important to include in investigations of the effects of stress.

Effects of Stress

Stress can be experienced in different ways, such as negative emotions, behavioral disruptions, and physiological reactions (Baum, Singer, & Baum, 1981; Grunberg & Singer, 1990; Baum, Gatchel, & Krantz, 1997; Park, Cambell, & Diamond, 2001; Bauer, Perks, Lightman, & Shanks, 2001). Similar to the effects of environmental enrichment, the effects of stress include biological and behavioral consequences. These categories of findings are consistent in both animal and human investigations. The duration, frequency, and intensity of a stressor profoundly influence the biological and behavioral response to stressors. Typically, the stressors that are longer in duration, occur more frequently, and are more intense, provoke more profound biological and psychological reactions. This section briefly presents an overview of biological and behavioral responses to stress.

Biological Effects of Stress

Challenges to an organism's survival can produce biological responses that range from activation of neurotransmitter systems involved in the HPA axis

to altering the growth and physiology of internal organs and organ systems (Kvetnansky, Weise, & Kopin, 1971; Keim & Siggs, 1876; Martijena, Cavlo, Vosolin, & Monlina, 1997; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Pham, Soderstrom, Henriksson, & Mohammad, 1997; Bielajew, Konkle, & Merali 2002; Bauer, Perks, Lightman, & Shanks, 2001; Elliott, Faraday, & Grunberg, 2003). Activation of the HPA axis is one of the most recognized biological responses of stress (DeVries, Glasper, & Catillion, 2003). Measuring the stress hormones related to the HPA axis (e.g., corticosterone [CORT], adrenocorticotropin hormone [ACTH], and corticotropin-releasing factor [CRF]) is the primary means for measuring the biological effects of stress. Acute and chronic stress increases levels of the stress hormones (Kant, Leu, Anderson, & Mougey, 1987; Brown & Grunberg, 1995; Bauer et al., 2001; Faraday, 2002; Bielajew, Konkle, & Merali 2002). In particular, Plasma Corticosterone levels have been reported to increase in response to a stressor and decrease in response to repeated exposure to a stressor in different experimental stress models (Bhatnagar & Meaney, 1995; Meaney, Aiken, Sharma, & Viau, 1992; Larsson et al., 2002; Belz et al., 2003).

There are also other physiologic differences as a consequence of stress. For example, rats exposed to 30 minutes of restraint for 14 days had significantly larger adrenal glands and higher basal levels of Corticosterone compared to animals that were not exposed to a stressor (Bauer et al., 2001). Male Sprague-Dawley rats exposed to daily 20 minute sessions of restraint for 14 consecutive days had decreased heart length, decreased left ventricle cavity width, and

increased septal wall thickness. Restraint stress decreased total heart blood volume in female Sprague-Dawley rats (Elliott, Faraday, & Grunberg, 2003). Stress exposure also induces dramatic changes in immunity of laboratory animals indicating compromises in the immune system. For example, chronic stress may be associated with changes in glucocorticoid immunoregulation, which may alter the way lymphocytes respond to the steroid signaling in the immune system (Bauer et al., 2001). Stress clearly alters biologic function and structure in animals and humans.

Behavioral Effects of Stress

Animals exposed to stressors exhibit poorer performance on cognitive tasks compared with animals not exposed to stressors. Stress can interrupt attentional processing in rats as measured by pre-pulse inhibition of the acoustic startle reflex, but strain and gender differentially affect these responses (Acri, 1994; Faraday, 2002). With regard to learning and memory, stressed rats display inferior spatial learning and memory in the radial arm maze compared to non-stressed rats (Park, Campbell, & Diamond, 2001).

Stress also increases anxiety-like behaviors. In response to inescapable foot-shocks or immobilization, rodents decreased overall activity and increased defecation in an open field arena (Gamallo et al., 1988; van Dijken, Mos, van der Heyden, & Tilders, 1992; Faraday, 2002). Predator stress (i.e., exposure to a cat) impaired habituation to a novel environment in rats by increasing activity within the open field (i.e., Open-Field) (Park, Campbell, & Diamond, 2001). In studies using the elevated plus maze (EPM), exposure to an inescapable shock

decreased time in the open arms suggesting an anxiogenic response (Steenbergen, Heinsbroek, Van Hest, & Van de Poll, 1990; Marinjina et al., 1997; Kalinchev et al., 2002). It appears that stress alters activity in the Open-Field arena but the type of stressor may be important in determining how stress alters activity (e.g., foot shocks and restraint/immobilization decreased activity, predator stressor increased activity).

Food consumption and body weight are two measures of an animal's health that also can be affected by stress. Rats that are crowded or experience changes in their housing environment decrease food consumption (Brown & Grunberg, 1995; O'Conner & Eikelboom, 2000). Stress can temporarily increase or decrease food intake. For example, electric shock and restraint decrease food consumption (Rickards, Job, & Boakes, 1997; Marti, Marti, & Armario, 1994; Zylan & Brown, 1996); exposure to repeated cold stress increases feeding (Kawanishi, Fukuda, Tamura, Nishijo, & Ono,1997); noise stressors increase (Rasbury & Shemberg, 1971; Wilson & Cantor, 1986) and decrease feeding (Krebs, Macht, Weyers, Weijers, & Janke, 1996). Pijlman, Wolterink, & Van Ree (2003) suggest that stress may influence the sensitivity of subjects to rewarding stimuli. They report that physical stress induced a long-term decrease in preference for saccharine and open field activity compared to control treatment. Further, the emotionally stressed animals increase open field behavior activity and saccharine preference.

Environmental Enrichment and Stress

Environmental enrichment and stress may interact to alter biological and behavioral consequences. Environmental enrichment appears to provide beneficial consequences, whereas stress often negatively impacts the organism. Stress can be experienced in different ways, such as negative emotions, a disruption of behaviors, and physiological reactions (Baum, Singer, & Baum, 1981; Grunberg & Singer, 1990; Baum, Gatchel, & Krantz, 1997). Environmental enrichment enhances performance and significantly reduces emotionality (Haywood & Tapp, 1966; Kaler & Freeman, 1994; Joseph, 1999; Grindrod & Cleaver, 2001). Enriched environments produce more rapid adaptation to a novel environment (Varty et al., 2000) and superior information processing (Woodcock & Richardson, 2000).

Few experiments have examined enrichment and stress together in rats. Larsson, Winblad, and Mohammed (2002) examined the behavioral effects of enrichment and pre-exposure to a stressor. The enriched housing consisted of eight rats together in cages containing wheels, ladders, tunnels, and balls. Non-enriched animals were singly housed with no exposure to extra stimuli in their cages. After 30 days of differential housing, and prior to behavioral testing, the animals were moved to individual cages. Two days before behavioral testing, animals were exposed to one of three conditions: no stress, mild stress (passive avoidance box without electric shock), or a more powerful stress (passive avoidance box with electric shock). Re-exposure to the passive avoidance box served as the experimental stressor in the two stress conditions. Enrichment and

pre-exposure to a passive avoidance box initially increased locomotor activity for the first 5 minutes of Open-Field observations and then decreased activity compared to the non-enriched and non-stress exposed animals. These findings may suggest that enrichment and stress have similar behavioral effects. Studies need to examine environmental influence and the simultaneous application of stress.

Gadek-Michalska and Bugajski (2003) examined the effects of pre-experimental handling, restraint, and social crowding on the hypothalamic-pituitary-adrenocortical (HPA) response to 10 minutes of restraint. The animals were housed in groups of seven per cage (i.e., social enrichment) for 10 days. The animals were then moved to individual housing prior to experimental testing. Short-durations of stress induced by handling, restraint, or social crowding reduced the rats' HPA axis responses to acute stress. For example, handling reduced the animals' corticosterone responses by 41.5%. These findings indicate changes in stress responses that suggest an attenuation as a result of enriched housing, but it is not clear whether stress responses were attenuated by housing environment or by pre-exposure to short-duration stress.

Schrijver et al. (2002) examined the effects of enrichment on one exposure to 20 minutes of restraint stress after almost 12 weeks of differential housing. The investigator reported that housing did not significantly alter basal and response levels of plasma ACTH and corticosterone to 20-minutes of restraint. However, Schrijver et al. (2002) reported that enriched rats had attenuated ACTH and plasma corticosterone responses to stress compared to

non-enriched rats. One methodological limitation of this study is that the impact of enrichment on stress was only accomplished after all behavioral measures had taken place. Therefore, the non-enriched animals also were exposed to some environmental enrichment because they were tested in several experimental measures prior to being stressed (e.g., open-field arenas, water maze, light/dark box). Consequently, no definitive conclusions can be reached regarding environmental enrichment and the effects of stress from this experiment.

These experiments do not reveal a clear picture of behavioral effects of housing exposure and stress. The present experiment was designed to determine behavioral effects of housing and of stress that may be relevant to health.

SECTION II - DESCRIPTION AND VARIABLE RATIONALE

Independent Variables

Environmental Enrichment

Enriched environments refer to the amount of physical or social stimulation that is available in the environment. There are several different ways to manipulate and conceptualize environmental enrichment: neonatal handling (Meaney, Aitken, Sharma, & Viau, 1992), pretest handling (Schmitt & Hiemke, 1997), social enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), physical enrichment (Renner & Rosenzweig, 1986; Varty et al., 2000), and incorporation of natural environmental objects (Schrijver et al., 2002). Enriched

environments also vary in the amount of time animals are exposed to enrichment ranging from 12 days (Passineau, Green & Detrich, 2001; Elliott & Grunberg, 2004) to a year (Ickes et al., 2000). The most common enriched environments in animal research house 3 to12 rats in cages filled with toys and objects (e.g., pieces of wood, plastic bones, exercise wheels, balls, tunnels). This paradigm provides opportunities for social interaction and physical stimulation (Rosenzweig & Bennett, 1996; Woodcock & Richardson, 2000). Enriched environments differ from isolated environments in the number of animals per cage and the number of objects per cage (Rosenzweig & Bennett, 1996; Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998; Van Praag, Kempermann, & Gage, 1999; Varty, et al., 2000; Schrijver et al., 2002).

The present experiment provided subjects with social and physical enrichment for a total of 24 days. Figures 1 (Enriched) and 2 (Non-Enriched) provide pictures of the home cage environments in the present experiment. Detailed descriptions of housing conditions are provided in the methods section.

Stress Manipulation: Immobilization

Stress manipulation in animal experiments varies greatly (e.g., electric shock, crowding, cold water immersion, predator, intruder, or immobilization). A 20-minute immobilization or restraint was used in this experiment. Figure 3 provides a picture of the animal restrainer used in the present experiment. Short-term restraint (e.g., 15 – 30 minutes) is a widely used stress manipulation that is not painful and elicits behavioral and biological stress responses in rodents, including elevations in hypothalamo-pituitary-adrenocortical (HPA) hormones

(Kant, Leu, Andersen, & Mougey, 1987; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992; Plotsky & Meaney, 1993; Acri, 1994; Faraday, O'Donoghue, & Grunberg, 1999; Faraday, 2002).

Dependent Variables

The present experiment examined effects of enrichment on five dependent variables: Open-Field / Locomotor Activity, Elevated Plus Maze, Light/Dark Box, Plasma Corticosterone, Food Consumption, and Body Weight. Open-Field (i.e., horizontal and vertical activity) is a simple, widely used, measure of health, exploration, and habituation to a novel environment. In addition, Open-Field center time activity provides a behavioral index of stress - more time spent in the center of the test chamber is interpreted as less stress. Performance in the Elevated Plus Maze and Light/Dark Box provide additional behavioral indices of stress. The time spent exploring the open arms of the Elevated Plus Maze and the time spent exploring the light side of the Light/Dark Box are considered behavioral indices of stress – the more time in the open arms or on the light side are interpreted as less stress. Plasma Corticosterone levels provide a biological measure of stress. Corticosterone is a hormone that is directly involved in the stress response of animals - more stress is associated with increases in Plasma Corticosterone levels. Food Consumption and Body Weight provide information about health and health behaviors. This section provides a description of each dependent variable. Details describing the equipment and exact procedures are presented in the Methods section of this paper.

Open-Field Activity (OF)

Open-Field locomotion refers to an animal's behavior when placed in a non-home cage arena. The apparatus is an empty box with clear sides and a clear top that is used to measure the animals' activity in a novel environment (see Figure 4 for a picture of an Open-Field arena). Animal behaviors in the Open-Field have been used as measures of general locomotion, exploration, and anxiety or stress responses. Open-Field locomotion includes activity in the horizontal plane, activity in the center of the arena, and rearing or vertical activity. Level of activity and frequency of rearing behaviors reflect the extent to which animals habituate to a novel environment (Varty et al*.,* 2000; Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996). Habituation is a basic form of learning and refers to the progressive reduction in response to an initially novel stimulus when the stimulus is repeatedly presented (Varty et al., 2000). A decrease in overall activity or rearing behaviors is indicative of habituation to novel stimuli. No change in activity over time reflects deficient information processing. Deficiencies in processing novel information may decrease learning rates and interfere with an organism's ability to adapt effectively to its environment. Environmental enrichment has been reported to enhance a rat's ability to adjust and adapt to novel stimuli. Conversely, stress has been reported to change behaviors related to learning and memory in a manner consistent with disrupting informational processing. Open field activity provides a useful way to examine effects of enrichment and stress.

Open-Field and Enrichment

Animals raised in enriched environments exhibit reduced locomotor activity and reduced exploration over time (Varty et al*.,* 2000; Bowling *et al.,* 1993; Van Wass & Soffie, 1996; Paulus, Bakshi, & Geyer, 1998; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). In addition, enriched animals exhibit a more rapid decrease in activity in an open field arena compared with non-enriched animals. This change in activity is interpreted as an index of increased habituation to the novel environment and is believed to indicate enhanced learning (Varty et al., 2000). These reports suggest that environmental enrichment enhances ability to adjust and adapt to novel stimuli. In contrast, animals raised in non-enriched environments exhibit hyperactivity and decreased activity habituation. Environmental enrichment appears to improve information processing and adaptation to novel environments.

Open-Field and Restraint

Restraint stress has been reported to decrease Open-Field activity in rats (Galea, Wide, & Barr, 2001; Faraday, 2002). After 20 minutes of restraint, Open-Field activity was decreased in male Sprague-Dawley and male Long Evans rats, but only on the first day of stress. On four subsequent Open-Field sessions, stress did not appear to significantly reduce initial locomotor activity (Faraday, 2002). The adult, male rats appeared to have habituated when exposed to a repeated stressor. Lee, Tsai, and Chai (1986) reported that immediately following 1-hour restraint, activity and center time increased in mice. Increased center time has been interpreted as decreased anxiety and decreased center

time is interpreted as increased anxiety (Gamallo et al., 1986; Lee, Tsai, & Chai, 1986; Beck & Luine, 2002). Variations in the amount of restraint and the type of subjects used to investigate stress responses have provided differential results. In addition, it is not clear if these patterns of behavior seen in adult subjects also occur in adolescent rats. The effects of environmental enrichment are primarily studied using adolescent subjects. Therefore, to investigate the effects of environmental enrichment and stress, it is important to examine the effect of stress on the adolescent subject.

Elevated Plus Maze (EPM)

Elevated Plus Maze is widely used as an index of anxiety in rodent research (Pellow, Chopin, File, & Briley, 1985; Hogg, 1996; Kalinichev et al., 2002). The apparatus consists of four radiating platforms that are at right angles to each other. Two of the arms have high walls that enclose the platforms; two of the arms have no walls (see Figure 5 for a picture of an Elevated Plus Maze). Each subject is initially placed on an open-arm platform and time and entries into the open and closed platform arms are observed and recorded. This task does not require training, food or water deprivation, or aversive stimuli. The task is easy to conduct and typically takes 5 minutes to complete. A variety of species have been used in the Elevated Plus Maze, including rats (Pellow, Chopin, File, & Briley, 1985), mice (Lister, 1987), guinea pigs (Rex, Fink, & Marsden, 1994), and wild voles (Hendrie, Eilam, & Weiss, 1974). The Elevated Plus Maze is bidirectionally sensitive to anxiety manipulations and anxiety-like responses. Therefore, Elevated Plus Maze is sensitive enough to detect both increases and

decreases in anxiety. The two primary indices of anxiety in the Elevated Plus Maze are the percentage of time spent on the open arms and the percentage of entries into open arms.

Elevated Plus Maze and Enrichment

Few studies have examined enrichment and Elevated Plus Maze performance. Schmitt and Heimke (1998) reported that handling (a simple form of enrichment) resulted in subjects spending more time in the open arms of the maze, interpreted as a reduction in anxiety. Handling decreased overall activity but did not significantly affect the number of transitions from the open to closed arms of the Elevated Plus Maze. Santucci et al. (1994) reported that handling neonatal rats decreased anxiety according to Elevated Plus Maze performance indexed by more time spent in the open arms of the Elevated Plus Maze and more transitions between open and closed arms of the maze.

Elevated Plus Maze and Restraint

Stress has been reported to increase anxiety behaviors in the Elevated Plus Maze in rodents (Wigger & Neumann, 1999; Mcintosh et al.,1999; Kalinichev et al., 2002). Marinjena, Calvo, Volosin, and Molina (1997) restrained rats for 15 minutes, tested them 24 hours later on the Elevated Plus Maze, and reported an anxiogenic profile (i.e., less time in the open arms of the maze). Similar results were reported following a 2-hour restraint stressor with a 24-hour delay (Padovan, Del-Bel & Guimaraes, 1996; Mendonca & Guimaraes, 1998).

Light/Dark Box (L/D Box)

The Light/Dark Box is a more complex behavioral measure of anxiety. This task examines the behavior of animals in a two-chambered box consisting of a dark side and a brightly lit side (see Figure 6 for a picture of a of Light/Dark box). Rodents are nocturnal animals and prefer dark places. Rodents are initially placed in the less-preferred, lit side of the box, and subsequent behavior is observed. The amount of time spent on the lit side and the number of crosses between the light and dark sides of the box provide indices of anxiety. Less time in the light side and fewer number of side crossings are interpreted as anxiety (Gentsch et al., 1982; Zimmerman et al., 2001). Similar to the Elevated Plus Maze, the Light/Dark task does not require training, food or water deprivation, or other aversive stimuli.

Light/Dark Box and Enrichment

Few experiments have investigated enrichment and the Light/Dark box. Schrijver, Bahr, Weiss, and Wurbel (2002) reported that isolation-reared rats took more time to enter the dark side of the box and that enriched rats crossed into the light at a higher rate and habituated faster that non-enriched animals. Robbins et al. (1996) and Hall (1998) reported that isolation-reared rats spent less time than group-reared rats in the light side of a Light/Dark box, and interpreted this difference as indicating greater anxiety for the isolation-reared rats.

Light/Dark Box and Restraint

Few experiments have studied restraint stress and Light/Dark activity. Carli and Samanin (1988) reported that a single 2-hour restraint session significantly reduced the number of transitions between the light and dark sides of the box, as well as the amount of time spent in the lit side of the box (i.e., the aversive side) in adult male rats. Cancela, Bregonzio, and Molina (1994) replicated these results. Additionally, these investigators reported that previous exposure to persistent restraint (i.e., 2-hours of restraint daily for 7 days) produced an anxiolytic profile (i.e., increased the number of transitions between the light and dark sides of the Light/Dark Box and more time was spent in the light side of the box).

Plasma Corticosterone (CORT)

The hypothalamic-pituitary-adrenal (HPA) axis is involved in stress responses. HPA activity is reflected by plasma concentrations of several biochemicals, including corticosterone (CORT) (Selye, 1973; Hennessy, 1997; Pham et al., 1999; Belz, Kennell, Czambel, Rubin, & Rhodes, 2003). Investigations that examine biological markers of stress routinely examine levels of Plasma Corticosterone (Brown & Grunberg, 1995; Faraday, 2002; Larsson et al., 2002; Belz et al., 2003).

Plasma Corticosterone and Enrichment

The effects of enrichment on Plasma Corticosterone are not clear. Several studies report differences in Plasma Corticosterone between enriched

and non-enriched subjects (Van de Weerd et al., 1997; Pham et al., 1999; Larsson et al., 2002). However, the direction of these differences has not been consistent. Isolation-rearing has been found to increase (Gamallo et al., 1986), decrease (Sanchez et al., 1995), and have no effect on plasma corticosterone levels (Holson, 1991). In animal studies of social crowding, male Sprague-Dawley rats housed in groups had significantly higher Plasma Corticosterone levels than males housed alone (Brown & Grunberg 1995; Brown & Grunberg 1996). Belz et al. (2003) reported that rats reared in isolation with toys had significantly lower levels of corticosterone.

Plasma Corticosterone and Restraint

Restraint results in elevated stress hormones including Plasma Corticosterone (Kant, 1983; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992, Acri, 1994; Faraday 2002). Bauer, Lightman, and Shanks (2001) reported that one 30-minute session of restraint significantly increased Plasma Corticosterone in male, Sprague-Dawley, rats. These investigators reported that increased Plasma Corticosterone levels also were evident after repeated sessions (i.e., 30 minutes of restraint daily for 13 days). Additionally, Plasma Corticosterone was increased by a 30-minute immobilization completed daily for 5 days (with 2 day rest periods inbetween) (Ricart-Jane et al., 2002).

Food Consumption (FC) and Body Weight (BW)

Feeding and body weight are relevant to many physical and mental health conditions (e.g., anxiety, cancer, cardiovascular diseases, depression, diabetes,

eating disorders, obesity). Food Consumption and Body Weight are widely used in rodent experiments and they can be measured repeatedly in the same animals. In addition, Food Consumption and Body Weight are face-valid measures used with humans and animals (Brown & Grunberg, 1995; O'Conner & Eikelboom, 1999; Faraday, 2002). Food Consumption and Body Weight provide indices of the animal's state of health and, therefore, were included in the present experiment.

SECTION III - HYPOTHESES, METHODS, & RESULTS

This experiment examined the behavioral effects of environmental enrichment and stress in male adolescent rats. Some investigations have suggested that environmental enrichment may reduce responses to stress (Belz et al., 2003, Plotsky & Meaney, 1993), yet few studies have directly examined the effects of enrichment on the stress responses and these studies have reported mixed results (Larsson et al., 2002; Schrijver et al., 2002). The present experiment included two specific aims: (1) to examine effects of environmental enrichment on behaviors that index health, activity, and levels of stress, and (2) to determine whether environmental enrichment reduces effects of stress on the behaviors examined under Specific Aim 1.

This experiment makes two important contributions to previous investigations of enrichment and stress. The first contribution to previous research is that this experiment combined biologic and behavioral measures to provide convergent data to support the interpretation of results. Only one other investigation of enrichment and stress has examined behavioral and biological

variables together. Schrijver et al. (2002) examined the effect of enrichment on the biological response to stress, however only after all behavioral testing was completed. Therefore, the effect of environmental enrichment to impact the animals' behavioral responses to stress was not examined.

Second, this investigation included dependent variables that have not yet been used to examine enrichment and stress concurrently. Larsson et al. (2002) examined the behavioral effects of enrichment and stress on Morris Water Maze and Open-Field. To this author's knowledge, the present study is the first to use the Elevated Plus Maze and Light/Dark Box to examine the behavioral effects of enrichment and stress together.

Hypotheses

Specific Aim #1: Environmental Enrichment and Behavior

Hypothesis 1

Rats in the environmental enrichment condition will exhibit enhanced habituation and less activity in open-field arenas compared with rats in the non-enriched condition.

Rationale

Enrichment-reared rats exhibit enhanced learning compared with isolation-reared rats (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Varty et al., 2000).

Hypothesis 2

Rats in the environmental enrichment condition will spend more time in the open arms of the Elevated Plus Maze compared with rats in the non-enriched condition.

Rationale

Isolation-reared rats were more anxious or fearful than group-reared controls based on Elevated Plus Maze performance (Robbins et al., 1996; Hall, 1998). Neonatal handling and environmental enrichment together indicated that enrichment decreased anxiety as indexed by Elevated Plus Maze performance (Santucci et al., 1994).

Hypothesis 3

Rats in the environmental enrichment condition will spend more time on the light side of the Light/Dark Box than rats in the non-enriched condition.

Rationale

Isolation-reared rats were more anxious or fearful than group-reared controls based on Light/Dark Box performance (Robbins et al., 1996; Hall, 1998). Neonatal handling and environmental enrichment together indicated that enrichment decreased anxiety as indexed by Elevated Plus Maze performance (Santucci et al., 1994).

Specific Aim #2: Environmental Enrichment and Stress

Hypothesis 1

Stress will increase Open-Field activity and enrichment will attenuate this effect, such that: Not Enriched Stressed > Not Enriched Not Stressed $\geq$ Enriched Stressed > Enriched Not Stressed.

Rationale

Isolation-reared rats were more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998) and, immediately following 1-hour restraint, activity and center time increased in mice (Lee, Tsai, & Chai, 1986). Neonatal handling and environmental enrichment suggest that enrichment decreases stress responses (Santucci et al., 1994; Plotsky & Meaney, 1993).

Hypothesis 2

Stress will decrease time spent in the Elevated Plus Maze open arms and decrease time spent on the light side of Light/Dark box. Enrichment will attenuate this effect, such that: Not Enriched Stressed > Not Enriched Not Stressed $\geq$ Enriched Stressed > Enriched Not Stressed

Rationale

Stress increases anxiety behaviors of rodents in the Elevated Plus Maze (Wigger & Neuman, 1999; Mcintosh et al.,1999; Kalinichev et al., 2002). Isolation-reared rats were more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998). Neonatal handling and environmental

enrichment suggest that enrichment decreases stress responses (Santucci et al., 1994; Plotsky & Meaney, 1993).

Hypothesis 3

Stress will increase Plasma Corticosterone levels and enrichment will attenuate this effect, such that: Not Enriched Stressed > Not Enriched Not Stressed ≥ Enriched Stressed > Enriched Not Stressed.

Rationale

Restraint increases plasma levels of Plasma Corticosterone (Ricart-Jane et al., 2002; Bauer, Lightman, & Shanks, 2001). Rats exposed to novel cages have higher levels of stress hormones than rats exposed to familiar cages (Hennessy, 1997).

Methods

The purpose of the present experiment was to determine if rearing rats in enriched environments or non-enriched environments alters subsequent behaviors and responses to a moderate stressor.

Experimental Design and Determination of Sample Size

This experiment examined the effects of environmental enrichment and stress on male adolescent Sprague-Dawley rats. The experiment was conducted as a 2 (enriched or non enriched) x 2 (stress or no stress) factorial design with 12 subjects per cell. The sample size was determined based on previous reports using similar dependent measures and responses to

environmental enrichment and stress. Studies in the research literature reported statistically significant effects from cell sizes of 7 – 12 animals for enrichment (e.g., Van Praag et al., 1999; Passineau et al., 2001; Elliott & Grunberg, 2003) and 9 - 11 animals for stress effects (Schrijver et al., 2002; Faraday, 2002). Mering Kaliste-Korhonen and Nevalainen (2000) determined that 5 - 10 animals were needed to find statistically significant effects for enrichment on various biological measures (e.g., Body Weight , adrenal gland weights, fat adipose tissue).

Sample size determination analyses were conducted using the procedures of Keppel (1991); Keppel, Saufley, and Tokunaga (1992); and Cohen (1988). Estimates of effect size in the population were determined to provide 0.80 power by calculating an estimated omega squared (ϖ^2) according to the formula:

$$\varpi^2_A = \frac{\sigma^2_A}{(\sigma^2_A + \sigma^2_{S/A})}$$

where σ^2_A refers to the estimated population treatment effects, $\sigma^2_{S/A}$ refers to the estimated population error variance, and ϖ^2_A provides a measure of effect size that is relatively independent of sample size and is expressed as a proportion of the total variability ($\sigma^2_A + \sigma^2_{S/A}$) associated with the treatment or manipulation (σ^2_A)(Keppel *et al*., 1992).

Research Design and Methods

Subjects

The subjects were 48 male, adolescent (21 days old upon arrival) Sprague-Dawley rats from Charles River Laboratories. Male subjects were used to limit the possible complications in the interpretation of results that may be related to hormonal fluctuations associated with female estrus cycles. Adolescent animals were used to maximize the developmental impact of environmental environment and because of the investigator's interest in child/adolescent development. Sprague-Dawley rats were used because they are the most commonly used strain of outbred albino rats. Based upon baseline activity, twelve subjects were assigned to each of the four experimental treatment conditions five days after arrival to create comparable groups.

Housing

All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23^0 C and 50% relative humidity on a 12-hour reversed light/dark cycle (lights off at 0500 hours). The reversed light cycle was maintained so that behavioral measures could be accomplished during the animals' normal activity period. Animals were assigned to one of four housing conditions (Non-Enriched/Not Stressed [NENS], Non-Enriched/Stressed [NES], Enriched/Not Stressed [ENS], or Enriched/Stressed [ES]). In conditions ENS and ES, animals were housed in groups of three in larger polycarbonate cages

(46 cm x 36 cm x 20 cm). A variety of objects (durable dog and cat toys including colored textured balls, rings, and bones) were placed in the cage to provide physical and tactile stimulation (see Figure 1). Objects were removed 2-3x / week (or sooner if damaged) and replaced with new objects. The objects used, changing schedule, and cage dimensions were based on methods described in previous studies (Gardner et al., 1975; Varty et al., 2000; Elliott, 2004). In housing conditions NENS and NES, animals were single-housed in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm) with no additional objects (see Figure 2). This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Procedure

The experiment was conducted in three phases: Baseline (Phase I), Housing Only (Phase II), and Housing with or without Stress (Phase III). Table I presents the experimental Timeline. Phase I consisted of the first 5 days during which subjects were acclimated to the facility and to the equipment. During this phase, animals were housed individually in standard polycarbonate shoebox cages (40 x 20 x 20 cm). On day 1, animals arrived at the facility. On days 2-3, animals were handled once a day for 5 minutes. Handling reduces the stress associated with repeated handling that is necessary to conduct behavioral measures (Meaney et al., 1998). All animals then were acclimated to the Open-Field chambers (Day 4) to minimize contamination of responses by any stressful

effects of exposure to a novel situation (Faraday & Grunberg, 2000). Acclimation procedures do not affect later measurement of Open-Field habituation. On day 5, baseline Open-Field activity was measured and body weight was measured. These Baseline data were used to balance experimental groups. The experimental time line used during the acclimation and baseline period was based on previous studies in this laboratory in which these behavioral measures were used (Faraday et al., 1999; Cook, 2001; Faraday & Grunberg, 2000; Elliott & Grunberg, 2003).

Phase II was the 12-day Housing Condition Only Phase during which subjects were placed in either the environmental enrichment condition or the non-enriched condition. On Day 5, animals were assigned to one of the four treatment conditions (NENS, NES, ENS, or ES) and were placed in one of the two housing conditions on Day 6. Animals remained in the assigned housing conditions for the remainder of the experiment (i.e., a total of 24 days). The letter H and the number of days spent in this phase designate the experimental day during Phase II (e.g., H1 is the first day of the housing only phase).

Phase III was a 12-day Housing Condition with or without Stress Phase in which animals in the stress condition were immobilized in a non-painful plastic restrainer for 20 minutes each day (see Figure 3). The animals remained in their assigned housing condition throughout Phase II and Phase III. The letter S and the number of days spent in this phase designate the experimental days during Phase III (e.g., S1 is the first day of the stress phase).

All behavioral measures were conducted between 0530 and 0900 hours (at the beginning of the active/dark cycle). This period of time was used to maximize behavioral performance and activity.

Dependent Variables

Open Field (OF)

Open-Field activity was measured on Days 7, 17, 21, and 29 (i.e., Days H2, H12, S4, and S12). Open field activity was measured using an Onmitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH). Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas arena and a Plexiglas lid with multiple 3.5 cm diameter holes was placed on top of the arena. The lid ensures that subjects have adequate ventilation but cannot escape during data collection. A photocell array measured horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the arena floor. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity (see Figure 4 for a picture of an Open-Field arena). Data were transmitted to a computer via an Onmitech Model DCM-I-BBU analyzer. Once subjects were placed in the test arenas, the experimenter turned off the lights and left the room. The apparatus monitored animal activity continuously for a total testing period of 1 hour.

The interfaced software generates 21 sub-variables, including total distance in cm (a measure of overall activity) and horizontal and vertical activity

(measures of activity in the horizontal plane and exploratory activity, respectively). Horizontal activity, vertical activity, and center time were analyzed as measures of general activity and habituation, exploration, and anxiety, respectively. Groups that exhibit the greatest decrease in activity levels from baseline levels during the 1-hour testing session were interpreted as exhibiting the greatest habituation.

Elevated Plus Maze (EPM)

Elevated Plus Maze was measured on Day 19 (i.e., Day S2). The Elevated Plus Maze apparatus was built following the basic Plus Maze design of Pellow (1985). It has four arms radiating out from a central square platform and is looks like a large plus sign (also referred to as an x shaped). It is elevated 60 cm above the floor. Two of the four arms have opaque sidewalls (50 cm in height), while the remaining two arms have no walls or ledges. These two types of arms (enclosed and non-enclosed) are placed on opposing sides of the central platform, and are generally referred to as closed and open arms, respectively (see Figure 5 for a picture of an Elevated Plus Maze). Animals were placed in the center of the maze and allowed to explore the maze for 5 minutes. Environmental lighting was provided by a six-foot floor lamp with a 40-watt light bulb placed approximately 15-feet from the Elevated Plus Maze and pointed away from the apparatus. Elevated Plus Maze activity was recorded using a video camera and a commercially available software tracking system acquired from Actimetrics Corporation, Wilmetta, Illinois**.**

Light/Dark Box (L/D Box)

The Light/Dark Box was used on Day 23 (i.e., Day S6). The Light/Dark Box was built based on the description from Crawley, Skolnick, and Paul (1984). The light/dark test consists of a 16 in X 16 in X 12 in Plexiglas box separated into two chambers of equal proportions by a 16 in X 12 in partitioning wall (see Figure 6 for a picture of a Light/Dark box). The subjects are allowed to move between the chambers through a 5 in X 5 in hole located in the center of the partitions base. The front chamber (Light Side) is surrounded by white contact paper with the top uncovered. The back chamber (Dark Side) is surrounded by black contact paper with a hinged top cover that blocks light and allows access to the enclosed side for cleaning and animal removal.

The apparatus is situated inside an Open-Field arena - Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM[TAO]). Two arrays of photocells measure horizontal activity. The first array spans side-to-side and has 16 pairs of photocells, located on a plane 2 cm from the floor of the arena and spaced 2.5 cm apart from each other. The second array is configured identically to the first and runs front-to-back. Vertical activity is measured by a third array located on a plane 10.5 cm above the arena floor. Data were collected and transmitted to a computer via an Omnitec Model DCM-I-BBU analyzer. The data files are run through a VersaMax software program (Albany, New York) designed to filter data specifically for light/dark activity. Subjects were placed in the light side of the box facing the opening leading to the dark side. Once subjects were placed in the test arenas, the experimenter left

the room. Environmental light from ceiling lights provided the only illumination for the lighted side, and no light was provided for the dark side of the box. The apparatus monitored animal activity continuously for a testing period of 5 minutes.

Plasma Corticosterone (CORT)

Sample collection

On Day 30, animals were taken to another laboratory and anesthetized with Pentobarbital (50 mg/kg; IP injection volume 1 mg/kg) and were placed into a holding cage until unconscious as determined by observation and lack of reflex response to a tail pinch (approximately 3-5 minutes). The animals were then decapitated rapidly using a standard rodent guillotine (4.5 inch blade) and blood was immediately taken from the remaining trunk. The blood was placed in microcollection tubes and placed on ice for 20 minutes. The plasma was separated by centrifugation (3000 RPM for 14 minutes) and immediately placed into a - 80 ^{0}C freezer for later assay.

Plasma Corticosterone Extraction Process

Plasma corticosterone was assayed by an ImmuChem Double-Antibody radioimmunoassay (RIA) kit using ^{125} I-labeled corticosterone (ICN Biomedicals, Costa Mesa, CA). A limited amount of specific antibody is reacted with a fixed quantity of ^{125} I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increased as a function of the decreasing percentages of bound radioisotope-labeled corticosterone. A second antibody precipitates

antibody bound to antigen. The quantity of endogenous corticosterone was determined by measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting DPM into concentrations. All samples and standards were run in duplicate. This measure was included to estimate levels of stress, to verify effects of the stress manipulation, and to determine any enrichment effects on restraint stress.

Food Consumption (FC) and Body Weight (BW)

Food Consumption and Body Weight were measured at six different times: Days 5, 11, 17, 23, 26, and 30 (corresponding to Baseline, H6, H12, S3, S6, and S12).

Food Consumption

Food pellets were placed on the top of each cage and animals had continuous access to food. Food Consumption was determined by subtracting new food weights from previous food weights (e.g., subtracting Day H11 food weights from Day H6). When food was added, the new weight was recorded and this new weight was used in the next calculation. Two Food Consumption values were calculated for each animal during Phases II and III of the experiment.

Body Weight

Body Weight was measured at the same time as Food Consumption. Animals were removed from their cages and gently place on an electronic scale. To ensure accurate weight measurements (i.e., reduce measurement error) the

electronic scale automatically obtained multiple weight readings and provided an average of these readings. This procedure provided six body weight measures (one during Phase I, two during Phase II, and three during Phase III).

Results

Data Analytic Strategy

Subjects were assigned to experimental condition such that there were no significant differences in Open-Field activity or body weight among subjects in each experimental condition. Open-Field data were initially analyzed using separate multivariate analyses of variance (MANOVA) to examine the effects of enrichment and stress on locomotor activity (i.e., horizontal, vertical, and center time activity) during each experimental phase of the study (i.e., Phase II & III). In addition, within-session Open-Field activity was analyzed using repeated-measures ANOVA with enrichment and stress as the between-subjects factors and time as the within-subject factor. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVAs were performed separately for each Open-Field trial. Elevated plus maze, Light/Dark Box, and plasma corticosterone levels were analyzed with separate analyses of variance (ANOVA).

Repeated-measures ANOVAs were used to analyze food consumption (Food Consumption) and Body Weight. At Baseline (i.e., Phase I), there were no significant Body Weight differences among experimental conditions. Food Consumption and Body Weight were analyzed separately within each experimental phase of the experiment (i.e., Phases II and III). The average food

consumption was calculated from differences in food weights at the three time points during each experimental phase. Similar analyses were performed for body weight for Phases II and III of the experiment. Any significant main effects or interactions were examined using separate ANOVAs following the procedures of Keppel (1991).

Eta-squared values were used to determine the relative magnitude of enrichment effects for each group. Eta-squared is a measure of effect size that indicates the proportion of variance explained by a given independent variable. Eta-squared is the ratio of the between-groups sum of squares to the total sum of squares in the ANOVA (Cohen & Cohen, 1983).

Several strategies were used to minimize the probability of Type 1 error. First, the experiment was designed to provide adequate power (i.e., 0.80). Type I error is minimized when sample size supports adequate power (Keppel, 1991). In addition, only if overall analyses revealed a significant main effect or interaction were subsequent analyses performed. This strategy reduces the number of statistical tests performed (Keppel, 1991; Cohen & Cohen, 1983). All tests were two-tailed with significance determined by $p < 0.05$.

This section presents the experiment's statistical findings. The description includes supporting significant statistical values and refers to corresponding tables or figures. Detailed tables and figures of all statistical analyses are included in Section V.

Open Field Activity (Locomotion)

MANOVAs revealed significant differences between Enriched and Non-Enriched animals in total horizontal activity, vertical activity, and center time during Phase II and Phase III (see Tables 2a and 2b). These differences were examined using repeated-measures ANOVAs for each Open-Field trial. The results of the between-groups and the within-session analyses are presented separately for each Open-Field trial.

Phase I – Baseline

There were no significant differences in Open-Field activity at Baseline measurement. (see Tables 2a and Figures 7a - c).

Phase II – Housing Only Phase (Days H2 and H12)

The Phase II MANOVA revealed significant differences between Enriched and Non-Enriched animals in total horizontal activity, vertical activity, and center time (see Table 2a). Phase II repeated-measures ANOVAs, examining total horizontal, total vertical, and total center time from Open-Field 1 to Open-Field 2, revealed a significant time by enrichment interaction such that Enriched animal activity decreased over time (i.e., activity levels decreased from Baseline to Open-Field 2) but increased for Non-Enriched animals (see Tables 3a -c and Figures 7a - c). As a result of these statistically significant findings in the overall analyses, each Open-Field trial was analyzed separately. The results of the individual analyses are reported below.

Open-Field Trial 1 (Day H2)

Tables 4a - c present the details of the statistical analyses for Open-Field 1. Figures 8a - c present graphical depictions of the within-session horizontal, vertical, and center time activity for Open-Field 1.

Between Groups

Horizontal activity (F (1,46) = 59.18, $p < 0.001$), vertical activity (F (1,46) = 25.14, $p < 0.001$), and center time (F (1, 46) = 20.40, $p < 0.001$) were significantly lower for the Enriched animals compared to the Non-Enriched animals.

Within Session

Repeated-measures ANOVAs revealed significant main effects for time on horizontal activity (F (11, 506) = 37.69, $p < 0.001$), vertical activity (F (11, 506) = 43.41, $p < 0.001$), and center time (F (11, 506) = 6.70, $p < 0.001$) indicating that animal activity decreased over time in all conditions.

Open-Field Trial 2 (Day H12)

Tables 5a - c present the details of the statistical analyses for Open-Field 2. Figures 9a - c present graphical depictions of the within-session horizontal, vertical, and center time activity for Open-Field 2.

Between Groups

On trial 2, total horizontal activity (F (1,46 = 18.03, $p < 0.001$), vertical activity (F (1,46) = 11.02, $p < 0.05$), and center time (F (1, 46) = 6.15, $p < 0.05$) were significantly lower for Enriched animals compared to Non-Enriched animals.

Within Sessions

Horizontal activity (F (11, 506) = 83.76, $p < 0.001$), vertical activity (F (11, 506) = 66.04, $p < 0.001$), and center time (F (11, 506) = 6.67, $p < 0.001$) decreased over time for all animals. Time by enrichment interactions on horizontal activity (F (11, 506) = 2.79, $p = < 0.05$) and vertical activity (F (11, 506) = 2.02, $p < 0.05$) revealed a differential decrease in activity within the sessions. Specifically, Enriched animals horizontal and vertical activity deceased more rapidly compared to Non-Enriched animals. There was no significant interaction for center time.

Phase III – Housing With or Without Stress Phase (Days S4 and S12)

The Phase III MANOVA revealed that enrichment significantly decreased total horizontal activity, total vertical activity, and total center time during both phase III Open-Field trials (Open-Field 3 & 4) (see Table 2a). Stress significantly deceased total horizontal activity and total center time activity for Open-Field 3. There were significant interactions for enrichment and stress for total horizontal activity and total center time indicating that Enriched, Not Stressed animals had the least amount of total horizontal activity and total center time, followed by the Enriched, Stressed and Not Enriched, Stressed groups. The Enriched, Stressed

animals exhibited the most horizontal activity and center time. There were no main effects for stress and no enrichment by stress interactions for total activity measures during Open-Field 4.

Phase III repeated-measures ANOVAs, examining total horizontal, total vertical, and total center time from Open-Field 3 to Open-Field 4 revealed a significant main effect for time indicating that all activity decreased for all groups. Over time, a time by stress interaction for horizontal activity (F (1,44) = 4. 09, p < 0.05) indicated that total horizontal activity decreased differentially between the stressed and not stressed animals. Further, enriched animals displayed significantly less total horizontal activity (F (1,44) = 37.07, p < 0.001), total vertical activity (F (1,44) = 18.78, p < 0.001), and center time (F (1,44) = 34.63, p < 0.001) (see Tables 6a - c, and Figures 10a - c).

The results of the between-groups and the within-session repeated measures analyses are presented separately for Open-Field trial 3 and 4.

Open-Field Trial 3 (Day S4)

Tables 7a - c present the details of the statistical analyses for Open-Field 3. Figures 11a - c present graphical depictions of the within-session horizontal, vertical, and center time activity for Open-Field 3.

Between Groups

Horizontal activity (F (1,44) = 29.97, p < 0.001), vertical activity (F (1,44) = 14.15, p < 0.001), and center time activity (F (1, 44) = 25.31, p < 0.001) were significantly lower for Enriched animals compared to Non-Enriched animals. Stressed animals spent significantly less center time compared to Not Stressed

animals (F (1,44) = 4.60, p < 0.05). Significant enrichment by stress interactions for horizontal activity (F (1,44) = 8.38, p < 0.05) and center time (F (1,44) = 7.52, p < 0.05) revealed that Non-Enriched, Not Stressed animals displayed the most horizontal activity and center time, followed by Non-Enriched, Stressed, then Enriched, Stressed, and Enriched, Not Stressed had the least amount of horizontal activity and center time. There were no main effects or interactions for vertical activity.

Within Session

Horizontal activity (F (11, 484) = 103.41, p < .001), vertical activity (F (11, 484) = 66.57, p < 0.001), and center time activity (F (11, 484) = 18.59, p < 0.001) decreased during the session for all animals. The time by enrichment interactions were significant for horizontal (F (11, 484) = 7.42, p = < 0.001), vertical (F (11, 484) = 4.37, p = < 0.05), and center time activity (F (11, 484) = 8.00, p < 0.001) indicating that Enriched animals decreased activity more rapidly within session. A time by stress interaction for center time revealed that center time decreased more rapidly for Stressed animals compared to Not Stressed animals.

Open-Field Trial 4 (Day S12)

Tables 8a - c present the details of the statistical analyses for Open-Field 3. Figures 12a - c present graphical depictions of the within-session horizontal, vertical, and center time activity for Open-Field 3.

Between Groups

Horizontal activity (F (1,44) = 30.89, $p < 0.001$), vertical activity (F (1,44) = 18.93, $p < 0.001$), and center time activity (F (1, 44) = 19.05, $p < 0.001$) were lower for Enriched compared with Non-Enriched animals on Day S12. There were no significant main effects for stress or interactions between groups on this trial.

Within Session

Horizontal activity (F (11, 484) = 114.05, $p < 0.001$), vertical activity (F (11, 484) = 81.69, $p < 0.001$), and center time (F (11, 484) = 23.13, $p < 0.001$) decreased over time for all animals. Significant time by enrichment interactions for horizontal (F (11, 484) = 7.89, $p = 0.001$), vertical (F (11, 484) = 4.67, $p < 0.001$), and center time activity (F (11, 484) = 3.11, $p < 0.001$) revealed that Enriched animals decreased more within session compared to Non-Enriched animals. A significant time by stress interaction for vertical activity (F (1,44) = 2.14, $p < 0.05$) indicated that vertical activity decrease more rapidly in the Not Stressed animals compared to the stressed animals.

Open-Field *Results Summary*

There were no differences in activity levels during Phase I but Enriched animals were less active in the Open-Field activity chambers than were Non-Enriched during Phase II (Housing Only) and Phase III (Housing with or without Stress). Animals in the Enriched housing condition were less active during each of the four Open-Field trials and these animals decreased activity more rapidly within each Open-Field session compared with animals in the Non-Enriched

housing conditions. These differences in activity in the housing conditions are consistent with other studies (Bowling, Rowlett, & Bardo, 1993; Van Waas & Soffie, 1996; Varty et al*.,* 2000; Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). The activity levels for the Stressed animals compared to the Not Stressed animals were not consistent with previous reports in which stress generally decreased activity (Gamallo et al., 1986; Faraday, 2002; Beck & Luine, 2002)**.**

Elevated Plus Maze (EPM)

The percent of time in the open arms and the number of entries into the open arms of the Elevated Plus Maze provide indices of anxiety or stress (Santucci et al., 1994; Hogg, 1996; Cook, 2003). Increases in anxiety would be reflected by decreased time spent in open arms and fewer entries into the open arms. Conversely, increased time spent in open arms and higher numbers of entries into the open arms would indicate a decrease in anxiety. There were no significant main effects for enrichment or stress on this measure and there were no significant interactions (see Tables 9a - b., along with Figures 13a - b. for detailed Elevated Plus Maze results).

Light/Dark Box (L/D Box)

The percent of time spent in the light side of the Light/Dark Box provides another index of anxiety or stress (Crawley, 1981; Gentsch et al., 1982; Zimmermann et al, 2001). Five subjects' data were excluded from the final analysis (one NENS; two NES; one ENS; and one ES) because their values exceeded the group means by more than two standard deviations. Animals in

the Enriched housing condition spent significantly less time on the light side of the Light/Dark Box compared with animals in the Non-Enriched housing condition (F (1, 39) = 5.03, $p < 0.05$). There was no main effect for stress and no significant interactions (see Table 7 for summary of Light/Dark Box results; see Table 10a and Figures 14a - b for detailed Light/Dark results).

Corticosterone (CORT)

There were no significant effects for stress or enrichment (see Table 11 and Figure 15 for Plasma Corticosterone results).

Food Consumption (FC)

Food consumption was indexed by the change in amount of food available. Several times throughout the present experiment the amount of food consumed was calculated by subtracting the current amount of food available from the previous measurement (see Tables 12a - b and 13a - d along with Figures 16a - d, 17a - b for Food Consumption results).

Phase I – Baseline Phase

There were no Food Consumption measurements taken during baseline phase.

Phase II - Housing only Phase

Repeated measures ANOVA for Phase II revealed a significant main effect for time (F (1, 46) = 369.07, $p < 0.05$) indicating that feeding increased in both groups for each food consumption measurement. Further, there was a time

by enrichment interaction (F (1, 46) = 12.22, p < 0.001) such that Non-Enriched animals ate more than the Enriched animals (see Table 12a for Phase II Food Consumption results).

Change 1 (Days H2-H6)

There was no significant main effect for housing condition (see Table 13a and Figures 16a).

Change 2 (Days H6-H12)

There was a significant main effect for housing (F (1, 46) = 17.82, p < 0.001) indicating that animals in the Enriched housing condition ate less food than animals in the Non-Enriched housing condition (see Table 13b and Figures 16b).

Phase III - Housing With and Without Stress Phase

Repeated-measures ANOVA for Phase III revealed a significant main effect for time (F (1, 44) = 15.19, p < 0.001) indicating that feeding increased in all groups for each food consumption measurement. There were no main effects for enrichment or stress. A time by stress interaction (F (1, 44) = 5.43, p < 0.05) and a between groups main effect for stress (F (1, 44) = 4.11, p < 0.05) and stress by enrichment interaction (F (1, 44) = 28.77, p < 0.001) revealed that feeding decreased differentially between the stress groups. Non-Enriched Stressed animals ate significantly less followed by the other three groups; Enriched Not Stressed, Enriched Stressed, Non-Enriched Not Stressed animals, respectively (see Table 12b and Figures 17a - b).

Change 3 (Days S1-S6)

There were no significant main effects for housing or stress. There was a significant interaction of enrichment and stress (F (1, 44) = 18.45, $p < 0.001$) such that Non-Enriched, Not Stressed animals ate the most food followed by the Enriched, Stressed animals. The Enriched, Not Stress and Non-Enriched, Stressed Animals ate the least amount of food (see Table 13c and Figures 16c and 17a).

Change 4 (Days S6-S12)

There was a significant main effect for stress (F (1, 44) = 7.99, $p < 0.05$) indicating that stressed animals ate less than the animals that were not stressed. There was also an enrichment by stress interaction (F (1, 44) = 32.95, $p < 0.001$) that revealed that Non-Enriched, Not Stressed animals ate the most food followed by the Enriched, Stressed animals, Enriched, Not Stressed, then Non-Enriched, Stressed animals (see Table 13d and Figures 16d and 17b).

Body Weight (BW)

Body weight was measured six times during the experiment (one time during Phase I, two times during Phase II, and three times during the Phase III). Animals in the Enriched housing condition weighed approximately 6% less than animals in the Non-Enriched housing condition at the end of enrichment (see Tables 14a - b and Figures 18a - c for Body Weight results).

Phase I – Baseline Phase

There were no significant differences at Baseline (see Table 14a and Figure 18a).

Phase II – Housing only Phase

Repeated-measures ANOVA revealed a significant main effect for time during Phase II (F (3, 138) = 2235.66, $p < 0.001$) and no time by enrichment interaction indicating that Enriched and Non-Enriched animals gained weight. There were no significant differences between subjects during Phase II (see Table 14a and Figure 18a).

Phase III – Housing With and Without Stress Phase

During Phase III, within-subject analyses revealed that animals in the Enriched and Non-Enriched housing conditions gained weight over time (F (2, 88) = 465.58, $p < 0.001$) and there were no significant interactions of time, housing condition, or stress. A between-subjects main effect for housing indicates that the Enriched animals weighed significantly less than Non-Enriched (F (1,44) = 7.05, $p < 0.05$) (see Table 14b and Figure 18b).

SECTION IV - ASSESSMENT & DISCUSSION

Assessment of Study Hypotheses

Specific Aim #1: Environmental Enrichment and Behavior

Hypothesis 1

Rats in the Enriched condition will exhibit decreased activity and enhanced habituation in the open-field arena compared with rats in the Non-Enriched condition.

The first hypothesis was based on previous research indicating that environmental enrichment produces beneficial effects on varying measures of cognitive and behavioral activity (Gardner, Boitano, Mancino, & D' Amico, 1975; Smith, 1972; Varty et al., 2000).

This hypothesis was supported. There were no differences in Open-Field activity at baseline and environmental enrichment decreased overall activity and increased the rate of habituation in the Open-Field.

Hypothesis 2

Rats in the Enriched condition will spend more time in the open arms of the Elevated Plus Maze compared with rats in the Non-Enriched condition.

This hypothesis was based on previous research that reported less anxious animals would spend less time in the open arms of the Elevated Plus Maze. Research indicated that isolation-reared rats were found to be more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998)

and enrichment decreased behavioral and physiological reactions to stressors (Santucci et al., 1994; Plotsky & Meaney, 1993).

This hypothesis was not supported.

Hypothesis 3

Rats in the Enriched condition will spend more time on the light side of the Light/Dark Box than rats in the Non-Enriched condition.

This hypothesis was based on previous research that reported less anxious animals would spend less time in the open arms of the Elevated Plus Maze. Research indicated that isolation-reared rats were found to be more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998) and enrichment decreased behavioral and physiological reactions to stressors (Santucci et al., 1994; Plotsky & Meaney, 1993).

This hypothesis was not supported.

Specific Aim #2: Environmental Enrichment and Stress

Hypothesis 1

Stress will increase Open-Field activity and enrichment will attenuate this effect, such that: Non-Enriched Stressed > Non-Enriched Not Stressed $\geq$ Enriched Stressed > Enriched Not Stressed.

This hypothesis was based on reports that isolation-reared rats were more anxious or fearful than group-reared controls (Robbins et al., 1996; Hall, 1998) along with reports that neonatal handling and environmental enrichment suggest

that enrichment decreases stress responses (Santucci et al., 1994; Plotsky & Meaney, 1993).

This hypothesis was partially supported. Non-Enriched animals were more active than Enriched animals. Non-Enriched, Not Stressed animals exhibited the most activity, followed by the Non-Enriched, Stressed animals, and Enriched, Not Stressed. The Enriched, Stressed animals had the least amount of activity (the difference between Enriched animals was not statistically significant) (see Figures 10a - c).

Hypothesis 2

Stress will decrease time spent in the Elevated Plus Maze open arms and decrease time spent on the light side of Light/Dark Box. Enrichment will attenuate this effect, such that: Not Enriched Stressed > Not Enriched Not Stressed $\geq$ Enriched Stressed > Enriched Not Stressed

This hypothesis was based on reports that stress increased anxiety behaviors of rodents in the Elevated Plus Maze (Wigger & Neuman, 1999; Mcintosh et al.,1999; Kalinichev et al., 2002) and neonatal handling and environmental enrichment decreases stress responses (Santucci et al., 1994; Plotsky & Meaney, 1993).

This hypothesis was not statistically supported.

Hypothesis 3

Stress will increase Plasma Corticosterone levels and enrichment will attenuate this effect, such that: Not Enriched Stressed > Not Enriched Not Stressed $\geq$ Enriched Stressed > Enriched Not Stressed.

This hypothesis was based on reports indicating that restraint increases plasma levels of Plasma Corticosterone (Ricart-Jane et al., 2002; Bauer, Lightman, & Shanks, 2001) and that rats exposed to novel cages have higher levels of stress hormones than rats exposed to familiar cages (Hennessy, 1997).

This hypothesis was not supported.

Discussion

The purpose of this study was to examine if rearing rats in enriched environments altered responses to a moderate level of acute and chronic stress. The investigation had three experimental phases, baseline, housing only, and housing with or without stress. Animals were placed in Enriched or Non-Enriched environments for 24 days. After 12 days, half of the subjects in each housing condition were restrained daily for 20-minutes for the next 12 days. Six different measures were used to examine any differences between Enriched and Non-Enriched rearing environments: Open-Field locomotion, Elevated Plus Maze, Light/Dark Box, Food Consumption, Body Weight, and Plasma Corticosterone. Experimental results partially support the proposed hypotheses.

The Open-Field activity results supported the effectiveness of the environmental enrichment manipulation. The lower levels of activity and more rapid habituation in the Enriched animals parallel the findings from other

enrichment research (e.g., Rosenzweig & Renner, 1987; Varty et al., 2000). There are several questions regarding environmental enrichments effects on Open-Field activity that have not been addressed. For example, how long will enrichment decrease activity in open field after enrichment is discontinued or does enrichment differentially affect the animals based on gender or strain of rat?

Examining the effects of enrichment and stress, the support for enrichment to reduce anxiety was not as robust as expected. There were a total a eight variables that can be conceptualized as measures of anxiety: (1) Open-Field % time in the center of the box, (2) Light/Dark Box % time spent in the light side, (3) Light/Dark Box total activity, (4) Elevated Plus Maze % time spent in the open arms, (5) Elevated Plus Maze % entries into the open arms, and (6) Plasma Corticosterone. In addition, Food consumption (7) and Body Weight (8) have been reported as sensitive measures of an animal's response to stress (Brown & Grunberg, O'Conner & Eikelboom, 1999, Faraday 2000).

Three of the eight anxiety measures (Open-Field % time in the center of the box, Light/Dark Box total activity, and Food Consumption) indicated that enrichment decreased anxiety. Animals in the Enriched condition spent more time in the center of the Open-Field arena and had higher overall activity in the Light/Dark Box. In addition, food consumption was dramatically decreased by stress, and this effect seemed to be attenuated by enrichment (see Figures 17a - b).

Three of the measures (Elevated Plus Maze % time spent in the open arms, Elevated Plus Maze % entries into the open arms, & Plasma

Corticosterone) neither contradicted nor supported the hypothesis that enrichment can decrease anxiety. However, although not statistically significant, the Enriched animals did spend more time in the open arms as predicted and as previously reported by Santucci et al. (1994). Light/Dark Box % time spent in the light side suggests that enrichment might have increased anxiety. Schrijver, Bahr, Weiss, and Wurbel (2002) reported that Enriched rats crossed into the light at a much higher rate and habituated much faster that Non-Enriched animals. These investigators concluded that high levels of exploratory activity in the enrichment animals confounded the typical measures of anxiety (i.e., number of crossings and time spent in light side).

From a health psychology perspective, the decreased rate of weight gain in the Enriched animals is perhaps the most interesting finding of the current experiment. Food Consumption and Body Weight and are primary health concerns in American society today. An estimated 31% of American adults (≈59 million people) and 15% of American children (≈9 million people) are classified as overweight or obese (National Institutes of Health, 2004). An estimated 1.7 billion people worldwide are overweight or obese (National Institutes of Health, 2004). Feeding patterns and body weight also are reported concerns in numerous medical conditions including cancers, cardiovascular disease, diabetes, and eating disorders. In the present study, Food Consumption and Body Weight were used to monitor animal health. Enriched animals weighed approximately 6% less than Non-Enriched animals after 24 days of enrichment. This difference in body weight is meaningful considering that the National

Institutes of Health (2004) recommend that overweight individuals decrease their body weight by 10% to gain health benefits from losing weight.

There are several possible ways to explain the weight differences between the Enriched and Non-Enriched animals in the present experiment. One explanation might be that the animals differed in weight at the beginning of the experiment. However, baseline Body Weight measures clearly show that the treatment groups did not differ. Conversely, differences in food consumption can explain the weight differences. In this study, three of the four Food Consumption measurements indicated that Enriched animals ate less than Non-Enriched animals. However, the first measurement indicated that the Food Consumption difference was minimal and during the last Food Consumption measurement, also the same measure indicating the greatest difference in Body Weight, the Non-Enriched animals ate slightly more than did Enriched animals. It appears that Food Consumption is partially responsible for the Body Weight differences. It would be interesting to examine if a variety of foods may differentially affect environmental enrichment's effect to decrease feeding. For example, environmental enrichment may affect the consumption of more preferred foods or have no effect on feeding when more preferred foods are available to subjects. Perhaps, even with more preferred foods available, environmental enrichment may prevent the animals from gaining excess weight compared to animals reared in Non-Enriched environments.

Another possible explanation for the differences in body weights is that the Enriched animals engaged in more physical activity than the Non-Enriched

animals. The result would be a higher body weight for Non-Enriched animals. The Enriched animals actually engaged in less activity during Open-Field trials. Despite the fact that the Non-Enriched animals were more active in novel environments (i.e., open field), brief, casual, observations made during cage changing, weighing, feeding, and transportation suggest that the Non-Enriched animals were less active in their home cages. Home cage activity is a critical variable to examine given environmental enrichment's potential to slow weight gain. If animals are more active in their home environments, then that might explain any weight differences in that the more active animals should expend more energy and therefore will weigh less than animals who do not expend as much energy. Home cage activity needs to be carefully monitored before reaching any conclusion about this issue. No reported studies have examined and compared home cage activity levels between animals reared in Enriched and Non-Enriched environments.

Enriched animals' slower weight gain also might be attributed to illness. It is conceivable that the Enriched environment "crowded" the animals providing stress (Bowen & Grunberg, 1995) and competition for food decreased the animal's health causing lower body weight. However, the experimenter and animal husbandry personnel monitored the animal's health (e.g., daily evaluations of the condition and color of fur, clarity of eyes, and tail color). None of the animals were reported to the veterinarian for possible illness. Constant decreased activity also could index animal illness. However, there were no significant differences in activity levels between Enriched and Non-Enriched

animals during the Elevated Plus Maze or Light/Dark box. For example, similar activity was noted in the Elevated Plus Maze as indexed by the total number of crossings. Based on this evidence, it is extremely unlikely that illness caused the lower body weight in the Enriched animals.

One other explanation for the lower weight gain in Enriched animals may be that environmental enrichment alters the animals' metabolism in some way. Given the fact that environmental enrichment alters brain cytoarchitechture and functioning, it is conceivable that it may also affect the organism's peripheral physiology including metabolism. More research is needed to examine the mechanisms and extent of environmental enrichment to slow weight gain.

Other animal research suggests that environmental manipulations can result in feeding and body weight changes (Levitsky, 1970; Marti & Marti, 1994; Fiala, Snow, & Greenough, 1997; Mueller, Loft, & Eikelboom, 1997; O'Conner & Eikelboom, 2000; Lopak & Eikelboom. 2000). For example, alternating rats between individually housed and paired housing conditions can result in a 23% suppression in feeding for up to three days (O'Conner & Eikelboom, 2000). Another environmental manipulation, the introduction of a running wheel, has been reported to decrease *ad libitium* feeding by up to 40 % for 10 days (Bauman, 1992; Tokuyama, Saito, & Okuda, 1982). The methodology and purpose of these experiments differed from the present experiment but alternative housing conditions and exercise wheel access could be conceptualized as "environmental enrichment" in the same manner that enrichment was manipulated in this experiment. Given the alarming rate of

obesity in American and the ability of the environment to influence feeding and body weight, the extent to which environmental enrichment influences feeding and body weight is a clinically relevant topic to investigate.

Careful investigation of enrichment effects on physical activity, food choice, and long-term weight gain may provide valuable insights into how to approach the problem of obesity in our society. In four weeks, this study showed that enrichment could decrease weight gain by 6% compared to animals raised in Non-Enriched environments. In addition, enrichment decreased food consumption. It would be important to carefully examine if this decreased rate of weight gain would continue over a longer period of time or if enrichment alters the consumption of all foods or only specific types of food (e.g., bland foods, sweet foods, high caloric foods).

Limitations

There are three potential limitations of this experiment that should be mentioned. The apparent lack of significant stress response to restraint, the homogeneity of subjects (i.e., only male subjects of one strain of rat), and the use of dependent variables not typically used in enrichment paradigms may be viewed as potential limitations in the current study. These three limitations are discussed in this section.

The most puzzling finding was the ineffectiveness of restraint to produce significant differences in the stress responses of the animals. Throughout Phase III (housing with and without stress phase), there were no consistent significant main effects for stress to indicate that the restraint stressor actually resulted in

elevations of stress in the animals. There are several possible explanations for this apparent lack of stress effect.

First of all, research does not offer a clear picture of the effect of restraint on the measures used in this study. Past research supported the use of restraint as a moderate stressor; however, few studies have examined the effect of restraint immediately following immobilization. For example, in the Elevated Plus Maze, after one 10 or one 20-minute session of restraint, anxiogenic profiles were not produced and restraint actually increased open arm exploration (Falter, Gower, & Gobert, 1992). McBlane and Handley (1994) reported no effect for 15 minutes of restraint on anxiety during the Elevated Plus Maze but that 1-hour of restraint significantly decreased open arm exploration.

In addition, the effect of enrichment on Plasma Corticosterone also had produced inconsistent results. Several studies report inconsistent differences in Plasma Corticosterone between Enriched and Non-Enriched subjects. Isolation-rearing has been reported to increase (Gamallo et al., 1986), decrease (Sanchez et al., 1995), and have no effect on plasma corticosterone levels (Holson, Scallet, Ali, & Turner, 1991). In animal studies of social crowding, male Sprague-Dawley rats housed in groups under crowded conditions had significantly higher Plasma Corticosterone levels than males housed alone (Brown & Grunberg 1995; Brown & Grunberg 1996). Belz et al. (2003) reported that rats reared in isolation with access to toys in their cages had significantly lower levels of plasma corticosterone.

In the present experiment, the use of pentobarbital to anesthetize the animals before the sacrifice may have affected the animals' hormonal stress responses, rendering the assay inconclusive. Pentobarbital is a central nervous system depressant that can disrupt the regulation of bodily functioning. Greer & Rockie (1968) reported changes in plasma corticosterone levels in response to ether and insulin. It is possible that other anesthesias may alter the release of plasma corticosterone. Another experimental investigation of stress was being conducted in this laboratory at the same time as the current experiment and found altered plasma corticosterone levels in response to restraint stress.

Another possible explanation for a lack of stress effect is that the restraint was not a "potent" enough stress manipulation in adolescent rats. Restraint and stress has typically been examined in adult animals and little information is known about the adolescent animals response to restraint. It may be that 20 minutes of immobilization does not affect the male adolescent in the same way as the adult male. Twenty minutes of restraint may not be long enough to produce the hormonal stress response in adolescents, or adolescent male rats, and consequently the animals would not appear to be stressed when compared to non-stressed animals. In the present study, the levels of corticosterone were similar to the values of stressed animals in previous studies (Brown & Grunberg 1995; Brown & Grunberg 1996; Belz et al. 2003). This elevated level may suggest that the animals were stressed or that adolescent males may have higher basal levels of plasma corticosterone.

All of the subjects in this experiment were male, Sprague-Dawley rats to reduce the potential for confounding variables between subjects in this initial investigation. The differential effects of enrichment based on gender and rat strain may be important to examine. For example, there have been clearly established gender and strain effects on stress responses (Faraday, 2000) and differential effects of enrichment on recovery from brain injury depending on gender (Elliott, 2004). Therefore, it would be important to investigate potential differences in response to environmental enrichment based on subject gender and strain. An interesting question is whether there are gender or strain differences in feeding and body weight in response to environmental variables and stress.

A third limitation of the present study is the use of measures that have not been extensively used to examine environmental enrichment. Few enrichment studies have used measures other than the open field test and memory tasks. It is not clear if these measures are sensitive to the effects of environmental enrichment or if they are sensitive in ways that have not typically been considered. For example, providing physical enrichment may provide the animals with greater ability to explore their environment. If this were the case, one would expect that Non-Enriched animals would have more difficulty in tasks that require dexterity (e.g., Elevated Plus Maze). In fact, this may be evident in this experiment. During the Elevated Plus Maze trials, the Enriched animals did appear to perform better than the Non-Enriched animals. Approximately 20% (5

out of 24) of the Non-Enriched animals fell off the Elevated Plus Maze, whereas none of the Enriched animals fell off maze.

Future Directions

The effect of enrichment to decrease feeding and slow body weight gain has experimental and clinical relevance. There are several possible future directions that are important to investigate (e.g., the effect of enrichment to decrease body weight and alter food consumption with different foods). Examining if enrichment affects consumption of different types of foods (e.g., bland, sweet, or salty foods) could be clinically useful. The use of differential foods could be important for two reasons. First, the current obesity epidemic facing our nation has been largely attributed to overindulgence in "junk food" or "fast food." Second, examining different types of foods could provide valuable information regarding the extent of the body weight effect. For example, because junk food is preferred by rats (Winders & Grunberg, 1996), it would be interesting to determine how environmental enrichment affects junk food consumption. It has already been reported that the use of a running wheel is chosen when presented with the option of a running wheel or sucrose water (O'Conner & Eikelboom, 2000). It would be interesting to learn if enrichment could avert overindulgence in junk food, resulting in a healthier individual with regard to their weight and nutrient intake.

It is also important to investigate the persistence of enrichment effects after enrichment is terminated and the duration of enrichment needed to alter feeding and body weight. By using different durations of enrichment and then

removing the animals from the enrichment, it may be possible to determine if "some" enrichment is better than none or to examine how long the effects of an enriched environment persists. These questions are particularly intriguing because investigators often remove animals from enriched conditions prior to behavioral testing.

Questions regarding the extent to which gender, genetic strain, or differing levels of enrichment (physical only, social only, "super enriched" [many cage mates housed in a multi-level cage]) differentially affect body weights are also important to consider when examining clinical implications of enrichment. In the present study, a modest amount of enrichment produced a slower rate of weight gain. By increasing the amount of enrichment (e.g., more cage mates, larger cage, and more to do), it may be possible to increase the weight differential between Enriched and Non-Enriched animals. In addition, physical activity was not carefully investigated in the present study. The difference in weight gain may simply be the result of an increase in physical activity in the home cages of enriched animals. Enriched animals habituate faster to novel environments (as demonstrated by Open-Field data in this study and others) suggesting that enriched animals engage in less activity in their home cages after the environment is no longer novel. It is not clear what role physical activity has in the decreased rate of weight gain and, to date, no experiments examining home cage activity and environmental enrichment have been reported. Therefore, physical activity in home cages and measures of metabolism should be included in studies of enrichment, food consumption, and body weight.

Conclusions

The purpose of this study was to determine if rearing rats in Enriched or Non-Enriched environments altered behavioral responses in a variety of tasks that are affected by stress. In addition, the experiment sought to determine if enrichment could affect the behavioral and biological responses of adolescent rats to a moderate level of acute and chronic stress. The results indicate that environmental enrichment does have behavioral effects on the adolescent rat. The impact of enrichment on stress in adolescent male rats was inconclusive. It is possible that the enrichment in the present investigation was not a powerful enough manipulation to decrease the male Sprague-Dawley rat's experience of stress. Several factors that may have confounded this investigation of enrichment and stress were discussed.

The fact that enrichment significantly decreased the rate of weight gain is a potentially important finding with clinical application. Given the current state of obesity in the United States, the extent to which environmental enrichment can affect feeding and body weight appears to be meaningful area of research that requires more investigation.

SECTION V –FIGURES, TABLES, REFERENCES

Figures and Tables

Figure 1.
Enriched Environment

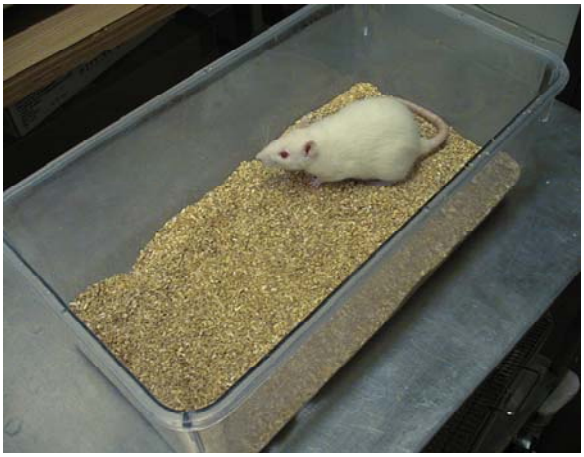


Figure 2.
Non-Enriched Environment

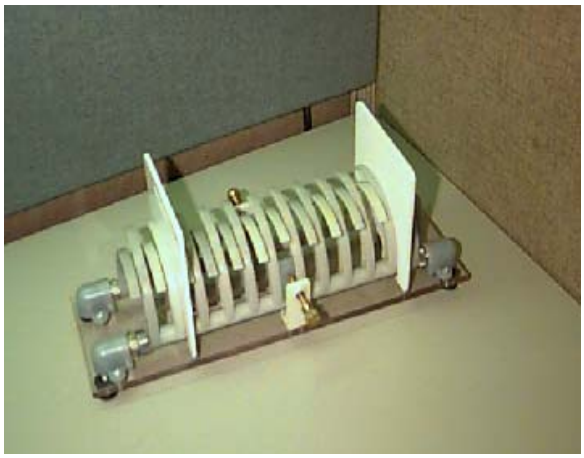


Figure 3.
Animal Restrainer

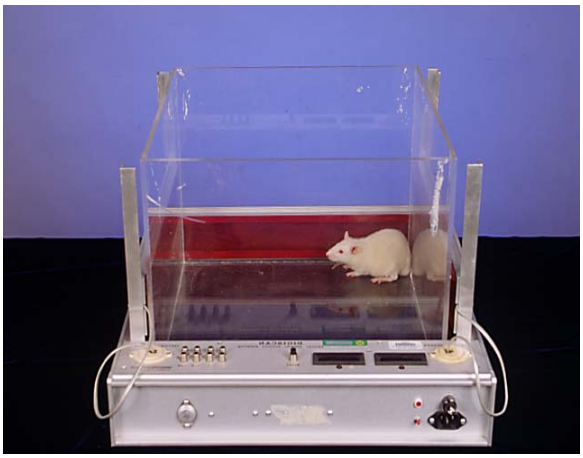


Figure 4.
Open Field Arena

Figure 5.
Elevated Plus Maze

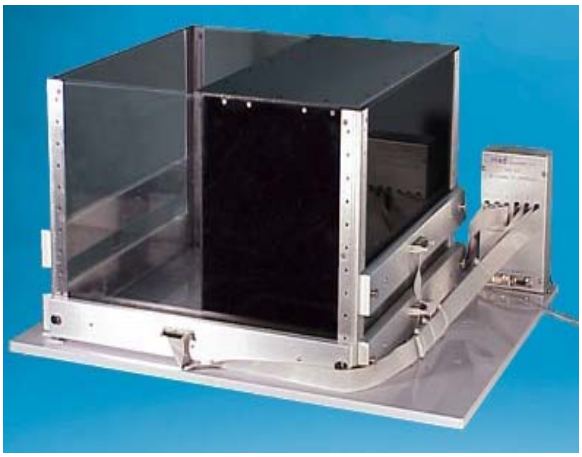


Figure 6.
Light/Dark Box

Table 1. Experimental Timeline	
Experimental Day	**Procedures**
Day 1	Animals Arrive
Day 2-3	Gentling
Day 4	OF Acclimation
Day 5	Baseline OF ; FC ; BW
Day 6 (H1)	Enrichment Phase
Day 7 (H2)	OF 1 ; FC ; BW
Day 8-10	No Measures
Day 11 (H6)	FC ; BW
Day 12-15	No Measures
Day 17 (H12)	OF 2 ; FC ; BW
Day 18 (S1)	Enrichment with Stress Phase
Day 19 (S2)	EPM ; FC ; BW
Day 21 (S4)	OF 3
Day 23 (S6)	L/D Box; FC ; BW
Day 24-27	No Measures
Day 29 (S12)	OF 4
Day 30	FC ; BW; Sacrifice ; CORT

H = Housing only Phase

S = Enrichment with or without Stress Phase

Initial Open-Field MANOVAs
Table 2a. Open-Field Phase I (Baseline) & Phase II (Open-Field 1& 2)

Tests of Between-Subjects Effects

Condition	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	TOTHACTB	112811.021	1	112811.021	.012	.913	.000	.051
	TOTVACTB	3024.188	1	3024.188	.134	.716	.003	.065
	TOTCTIMB	3084.813	1	3084.813	.206	.652	.004	.073
	TOTHACT1	584658760.083	1	584658760.083	67.313	.000	.594	1.000
	TOTVACT1	887264.083	1	887264.083	25.154	.000	.354	.998
	TOTCTIM1	311873.642	1	311873.642	20.400	.000	.307	.993
	TOTHACT2	253483188.021	1	253483188.021	17.841	.000	.279	.985
	TOTVACT2	783107.521	1	783107.521	11.017	.002	.193	.901
	TOTCTIM2	311841.400	1	311841.400	6.154	.017	.118	.680
Error	TOTHACTB	425288964.958	46	9245412.282				
	TOTVACTB	1038444.625	46	22574.883				
	TOTCTIMB	689441.353	46	14987.856				
	TOTHACT1	399540840.583	46	8685670.447				
	TOTVACT1	1622598.917	46	35273.889				
	TOTCTIM1	703240.211	46	15287.831				
	TOTHACT2	653555852.958	46	14207735.934				
	TOTVACT2	3269895.958	46	71084.695				
	TOTCTIM2	2330855.298	46	50670.767				

All statistical analyses computed using alpha = .05

ENRCHMT = Enriched vs. Non-Enriched Housing conditions
TOT = Total
HACT = Horizontal Activity
VACT = Vertical Activity
CTIM = Center Time

B = Baseline
1, 2, etc. = Open-Field trial number

Initial Open-Field MANOVAs

Table 2b. Open-Field Phase III (Housing with or without Stress)

Tests of Between-Subjects Effects

Condition	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	TOTHACT3	427189467.000	1	427189467.000	30.765	.000	.411	1.000
	TOTVACT3	2027052.000	1	2027052.000	14.576	.000	.249	.962
	TOTCTIM3	705165.842	1	705165.842	25.606	.000	.368	.999
	TOTHACT4	246219091.021	1	246219091.021	30.802	.000	.412	1.000
	TOTVACT4	1063860.750	1	1063860.750	18.931	.000	.301	.989
	TOTCTTIM4	459307.941	1	459307.941	19.054	.000	.302	.990
STRESS	TOTHACT3	68770044.083	1	68770044.083	4.953	.031	.101	.586
	TOTVACT3	157094.083	1	157094.083	1.130	.294	.025	.180
	TOTCTIM3	130614.900	1	130614.900	4.743	.035	.097	.568
	TOTHACT4	6426228.521	1	6426228.521	.804	.375	.018	.142
	TOTVACT4	17252.083	1	17252.083	.307	.582	.007	.084
	TOTCTTIM4	287.141	1	287.141	.012	.914	.000	.051
ENRCHMT * STRESS	TOTHACT3	75807160.083	1	75807160.083	5.459	.024	.110	.628
	TOTVACT3	202020.750	1	202020.750	1.453	.235	.032	.218
	TOTCTIM3	200712.400	1	200712.400	7.288	.010	.142	.752
	TOTHACT4	22697126.021	1	22697126.021	2.839	.099	.061	.378
	TOTVACT4	19764.083	1	19764.083	.352	.556	.008	.089
	TOTCTTIM4	4181.333	1	4181.333	.173	.679	.004	.069
Error	TOTHACT3	610960807.833	44	13885472.905				
	TOTVACT3	6119137.167	44	139071.299				
	TOTCTIM3	1211739.083	44	27539.525				
	TOTHACT4	351714571.750	44	7993512.994				
	TOTVACT4	2472633.000	44	56196.205				
	TOTCTTIM4	1060651.525	44	24105.716				

All statistical analyses computed using alpha = .05

Open-Field Phase II Results

Table 3a. Total Horizontal Activity - Baseline to Open-Field 2

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	545043498.028	1	545043498.028	35.499	.000	.436	1.000
Error	706280844.639	46	15353931.405				

Tests of Within-Subjects Contrasts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	1513530.375	1	1513530.375	.137	.713	.003	.065
TIME * ENRCHMT	121450505.042	1	121450505.042	11.024	.002	.193	.902
Error(TIME)	506771014.583	46	11016761.187				

Table 3b. Total Vertical Activity - Baseline to Open-Field 2

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	1046529.000	1	1046529.000	17.828	.000	.279	.985
Error	2700329.639	46	58702.818				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	45742.014	2	22871.007	.651	.524	.014	.156
TIME * ENRCHMT	626866.792	2	313433.396	8.926	.000	.163	.969
Error(TIME)	3230609.861	92	35115.325				

Table 3c. Total Center Time - Baseline to Open-Field 2

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	375482.988	1	375482.988	9.867	.003	.177	.868
Error	1750450.525	46	38053.272				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	39909.955	2	19954.978	.930	.398	.020	.207
TIME * ENRCHMT	251316.868	2	125658.434	5.859	.004	.113	.864
Error(TIME)	1973086.337	92	21446.591				

All statistical analyses computed using alpha = .05

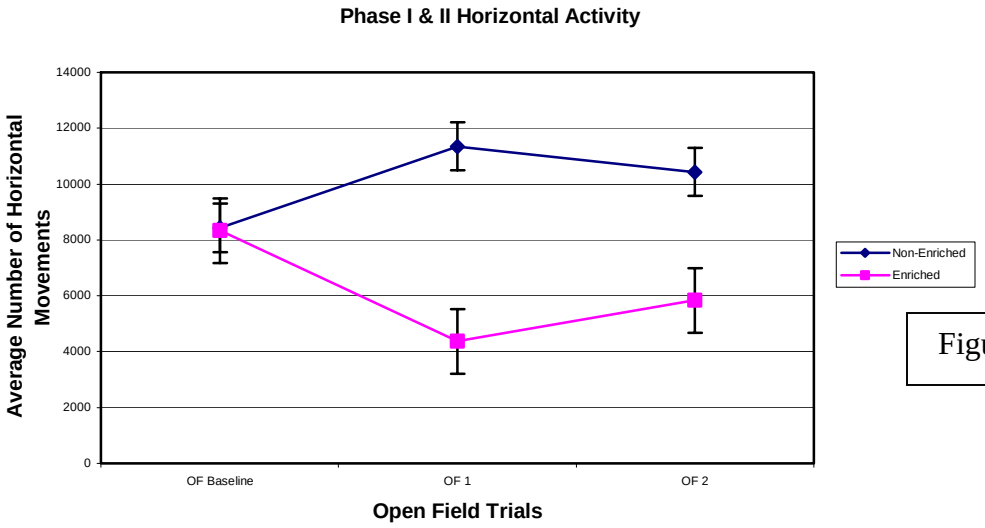


Figure 7a.

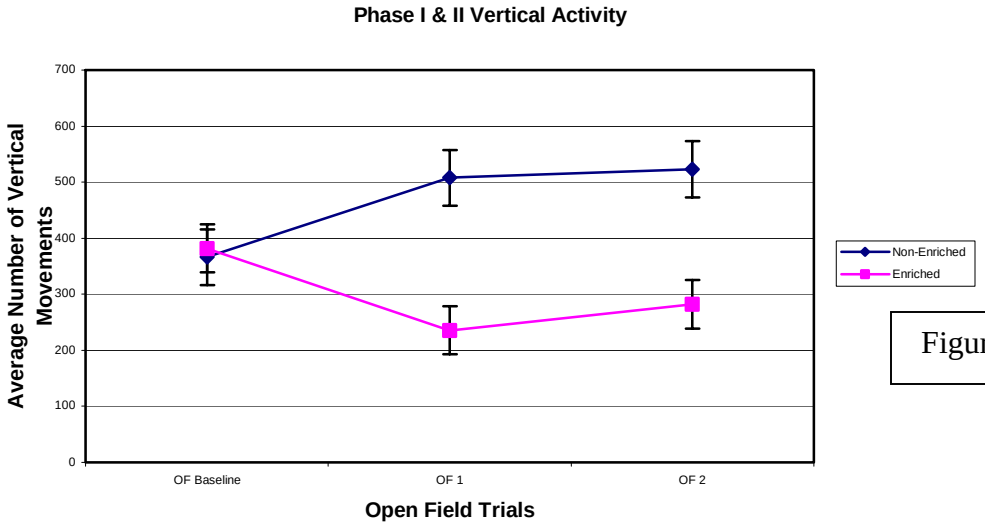


Figure 7b.

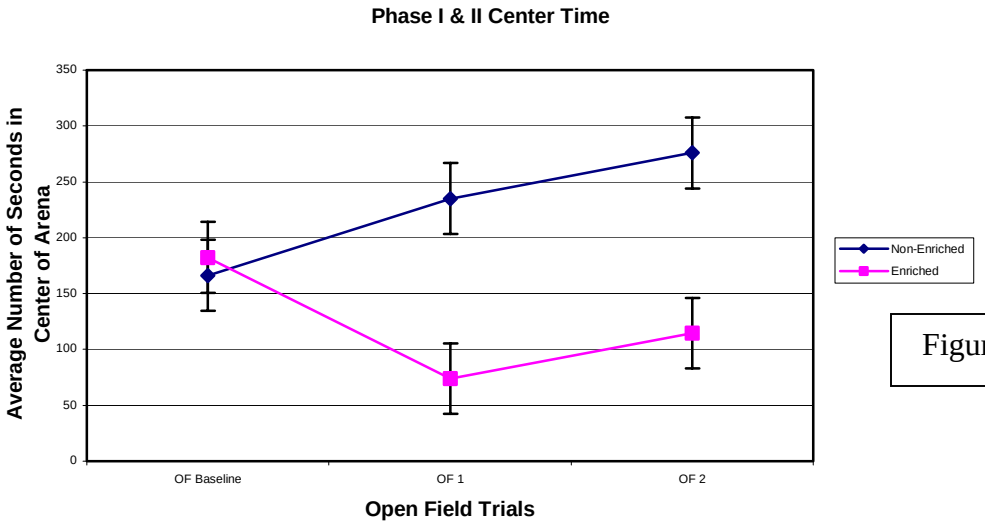


Figure 7c.

Open-Field Trial 1 Results
Repeated Measures ANOVAs
Table 4a. Open-Field 1 Horizontal Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	46335816.252	1	46335816.252	59.157	.000	.563	1.000
Error	36030653.691	46	783275.080				

Tests of Within-Subjects Contrasts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	93105177.714	11	8464107.065	37.688	.000	.450	1.000
TIME * ENRCHMT	3269879.852	11	297261.805	1.324	.207	.028	.715
Error(TIME)	113639638.851	506	224584.267				

Table 4b. Open-Field 1 Vertical Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	73938.674	1	73938.674	25.154	.000	.354	.998
Error	135216.576	46	2939.491				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	432794.667	11	39344.970	43.405	.000	.485	1.000
TIME * ENRCHMT	11308.326	11	1028.030	1.134	.332	.024	.630
Error(TIME)	458665.507	506	906.454				

Table 4c. Open-Field 1 Center Time

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	25989.470	1	25989.470	20.400	.000	.307	.993
Error	58603.351	46	1273.986				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	18874.713	11	1715.883	6.702	.000	.127	1.000
TIME * ENRCHMT	3803.040	11	345.731	1.350	.193	.029	.726
Error(TIME)	129544.190	506	256.016				

All statistical analyses computed using alpha = .05

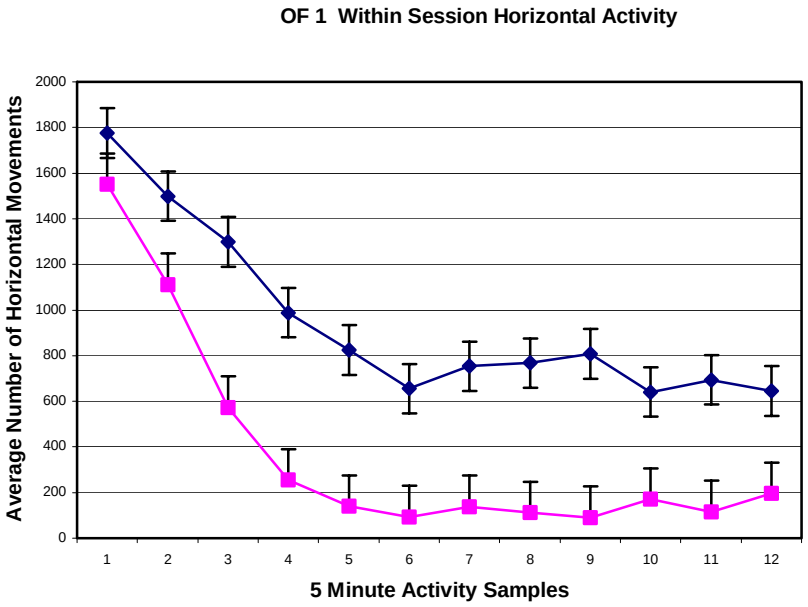


Figure 8a.

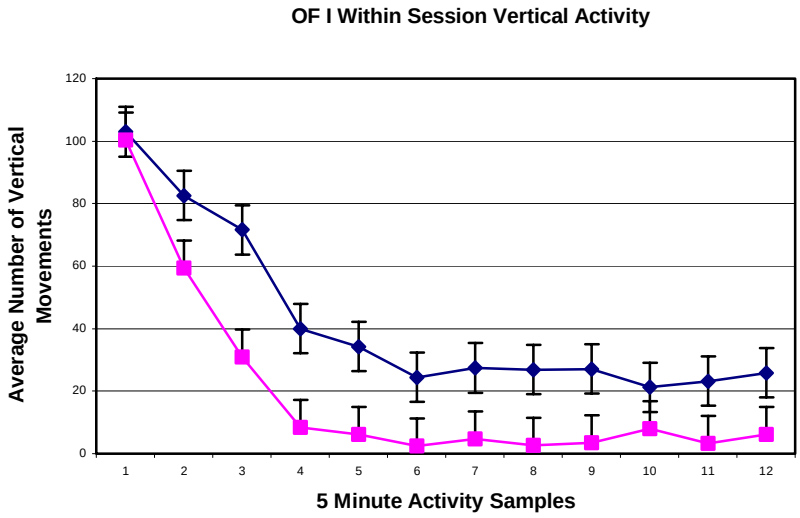


Figure 8b.

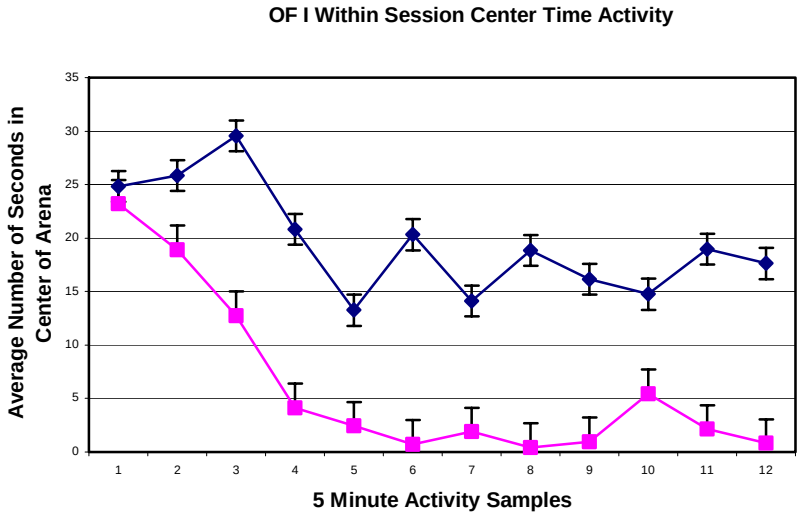


Figure 8c.

Open-Field Trial 2 Results
Repeated Measures ANOVAs
Table 5a. Open-Field 2 Horizontal Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	21992191.840	1	21992191.840	18.029	.000	.282	.986
Error	56110804.632	46	1219800.101				

Tests of Within-Subjects Contrasts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	149597626.306	11	13599784.210	83.761	.000	.646	1.000
TIME * ENRCHMT	4981551.326	11	452868.302	2.789	.002	.057	.981
Error(TIME)	82156428.535	506	162364.483				

Table 5b. Open-Field 2 Vertical Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	65258.960	1	65258.960	11.017	.002	.193	.901
Error	272491.330	46	5923.725				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	709181.727	11	64471.066	66.039	.000	.589	1.000
TIME * ENRCHMT	21739.894	11	1976.354	2.024	.024	.042	.910
Error(TIME)	493989.962	506	976.265				

Table 5c. Open-Field 2 Center Time

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	25986.783	1	25986.783	6.154	.017	.118	.680
Error	194237.941	46	4222.564				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	30896.411	11	2808.765	6.672	.000	.127	1.000
TIME * ENRCHMT	4702.047	11	427.459	1.015	.431	.022	.570
Error(TIME)	213028.894	506	421.006				

All statistical analyses computed using alpha = .05

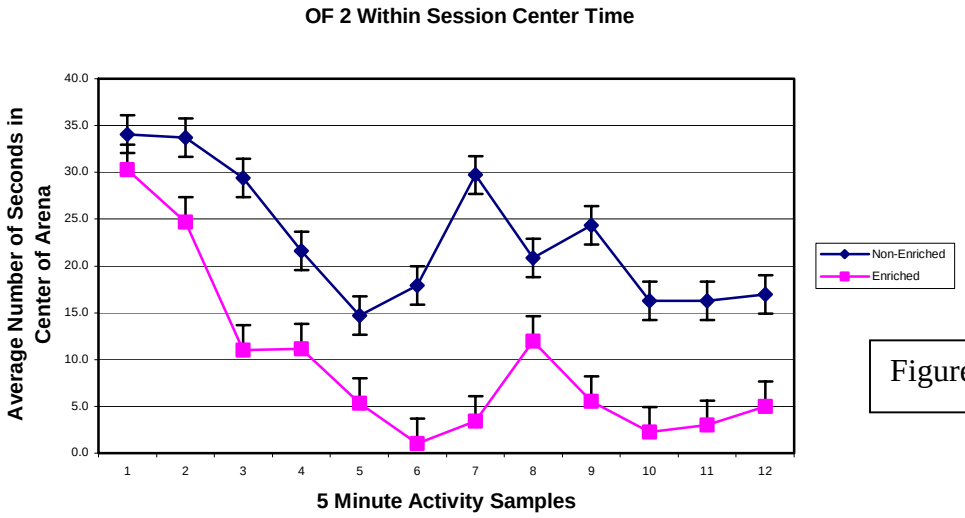


Figure 9a.

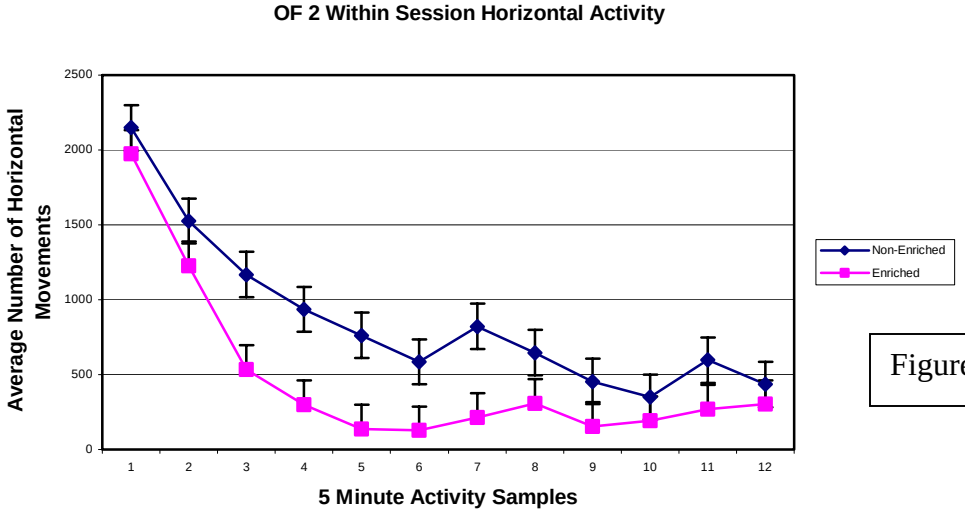


Figure 9b.

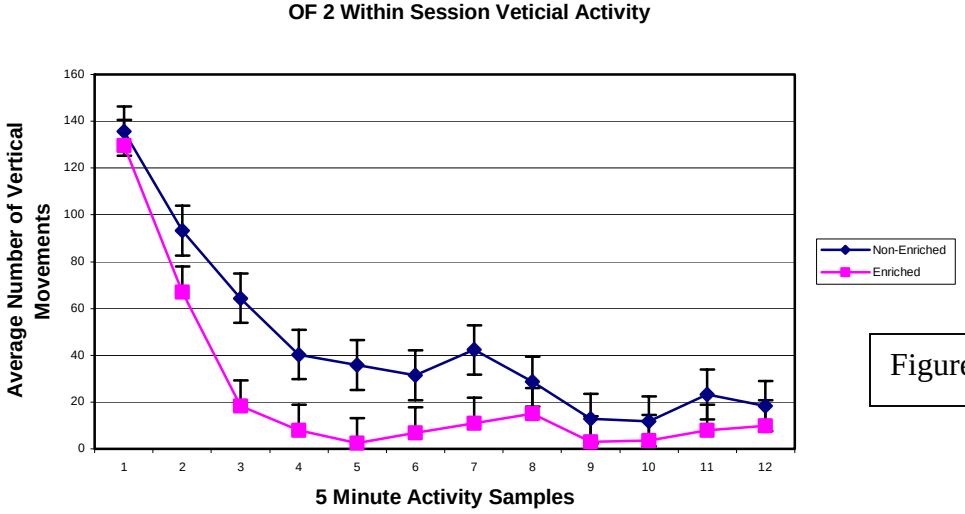


Figure 9c.

Table 6a. Total Horizontal Activity

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	135667282.594	1	135667282.594	33.541	.000	.433	1.000
TIME * ENRCHMT	12386221.760	1	12386221.760	3.062	.087	.065	.402
TIME * STRESS	16575957.094	1	16575957.094	4.098	.049	.085	.508
TIME * ENRCHMT * STRESS	7771971.094	1	7771971.094	1.921	.173	.042	.273
Error(TIME)	177969568.958	44	4044762.931				

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	661022336.260	1	661022336.260	37.065	.000	.457	1.000
STRESS	58620315.510	1	58620315.510	3.287	.077	.070	.426
ENRCHMT * STRESS	90732315.010	1	90732315.010	5.088	.029	.104	.597
Error	784705810.625	44	17834222.969				

Table 6b. Total Vertical Activity

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	747301.042	1	747301.042	21.493	.000	.328	.995
TIME * ENRCHMT	76953.375	1	76953.375	2.213	.144	.048	.307
TIME * STRESS	35113.500	1	35113.500	1.010	.320	.022	.166
TIME * ENRCHMT * STRESS	47704.167	1	47704.167	1.372	.248	.030	.209
Error(TIME)	1529888.917	44	34770.203				

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	3013959.375	1	3013959.375	18.779	.000	.299	.989
STRESS	139232.667	1	139232.667	.868	.357	.019	.149
ENRCHMT * STRESS	174080.667	1	174080.667	1.085	.303	.024	.175
Error	7061881.250	44	160497.301				

Table 6c.Total Center Time

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	85890.753	1	85890.753	4.668	.036	.096	.561
TIME * ENRCHMT	13125.065	1	13125.065	.713	.403	.016	.131
TIME * STRESS	71575.143	1	71575.143	3.890	.055	.081	.488
TIME * ENRCHMT * STRESS	73477.133	1	73477.133	3.994	.052	.083	.498
Error(TIME)	809515.001	44	18398.068				

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	1151348.718	1	1151348.718	34.630	.000	.440	1.000
STRESS	59326.898	1	59326.898	1.784	.188	.039	.257
ENRCHMT * STRESS	131416.600	1	131416.600	3.953	.053	.082	.494
Error	1462875.606	44	33247.173				

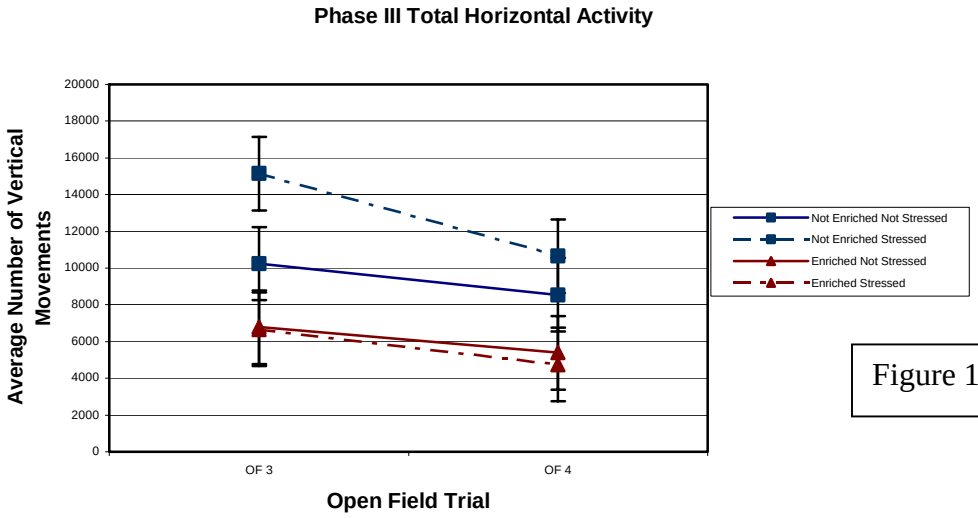


Figure 10a.

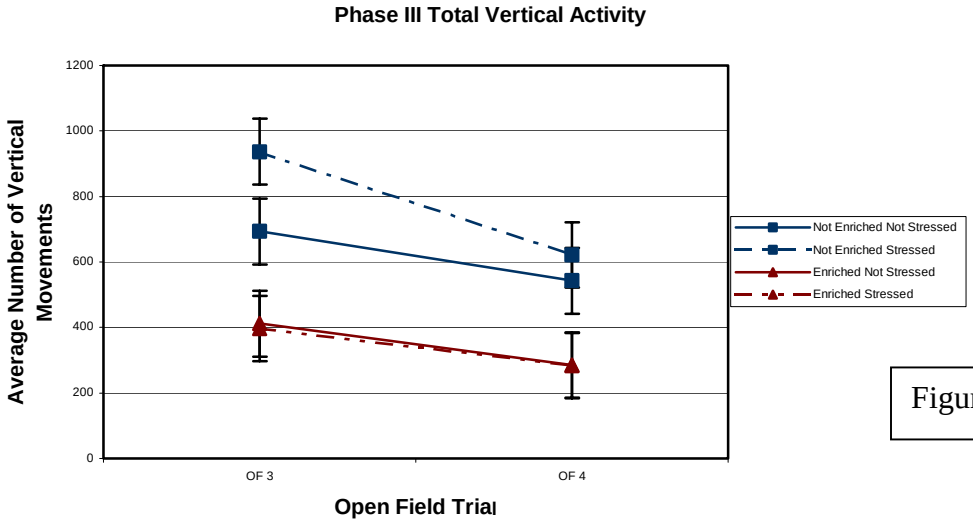


Figure 10b.

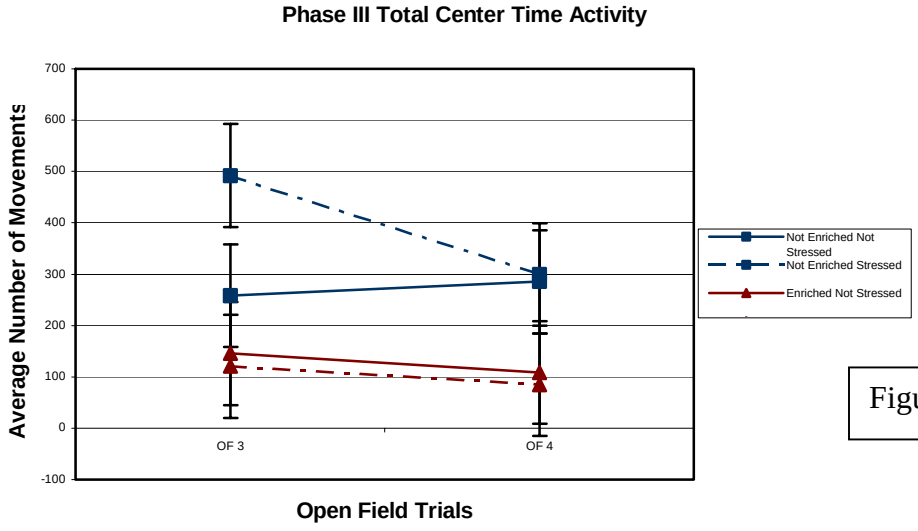


Figure 10c.

Table 7a. Horizontal Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	35107600.028	1	35107600.028	29.970	.000	.405	1.000
STRESS	3150920.840	1	3150920.840	2.690	.108	.058	.361
ENRCHMT * STRESS	9810990.063	1	9810990.063	8.375	.006	.160	.808
Error	51542738.208	44	1171425.868				

Tests of Within -Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	219048835.722	11	19913530.520	103.410	.000	.702	1.000
TIME * ENRCHMT	15718254.306	11	1428932.210	7.420	.000	.144	1.000
TIME * STRESS	3056583.993	11	277871.272	1.443	.150	.032	.761
TIME * ENRCHMT * STRESS	3453949.521	11	313995.411	1.631	.087	.036	.822
Error(TIME)	93203699.292	484	192569.627				

Table 7b. Vertical Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	3013959.375	1	163081.361	14.146	.000	.243	.957
STRESS	139232.667	1	11502.563	.998	.323	.022	.165
ENRCHMT * STRESS	174080.667	1	18746.174	1.626	.209	.036	.239
Error	7061881.250	44	11528.793				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	747301.042	11	128258.134	66.570	.000	.602	1.000
TIME * ENRCHMT	76953.375	11	8424.497	4.373	.000	.090	1.000
TIME * STRESS	35113.500	11	1850.199	.960	.482	.021	.541
TIME * ENRCHMT * STRESS	47704.167	11	1852.310	.961	.481	.021	.541
Error(TIME)	1529888.917	484	1926.669				

Table 7c. Center Time

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	1151348.718	1	57896.380	25.314	.000	.365	.998
STRESS	59326.898	1	10513.084	4.597	.038	.095	.555
ENRCHMT * STRESS	131416.600	1	17193.766	7.518	.009	.146	.765
Error	1462875.606	44	2287.114				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	79715.064	11	7246.824	18.590	.000	.297	1.000
TIME * ENRCHMT	34305.863	11	3118.715	8.000	.000	.154	1.000
TIME * STRESS	11789.153	11	1071.741	2.749	.002	.059	.979
TIME * ENRCHMT * STRESS	8277.018	11	752.456	1.930	.034	.042	.893
Error(TIME)	188670.719	484	389.816				

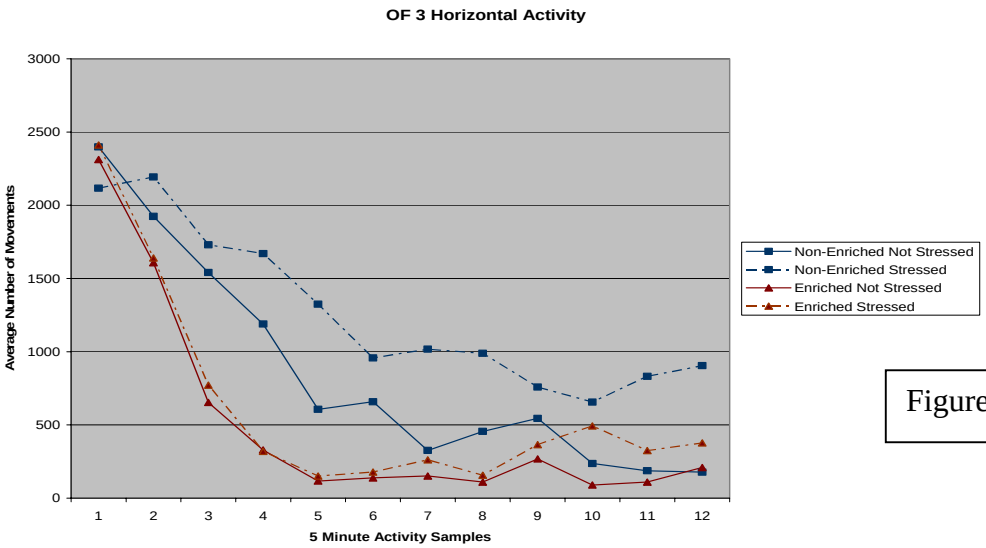


Figure 11a.

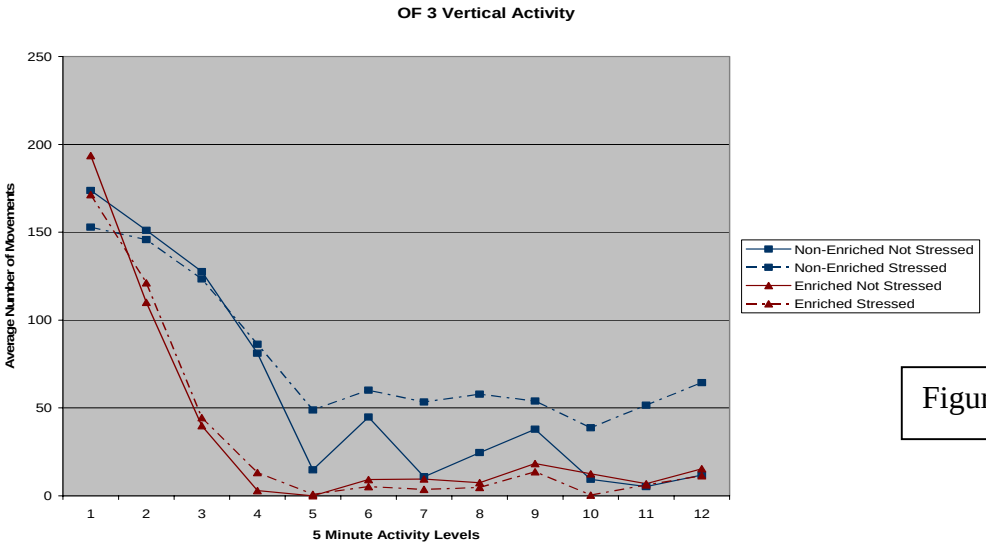


Figure 11b.

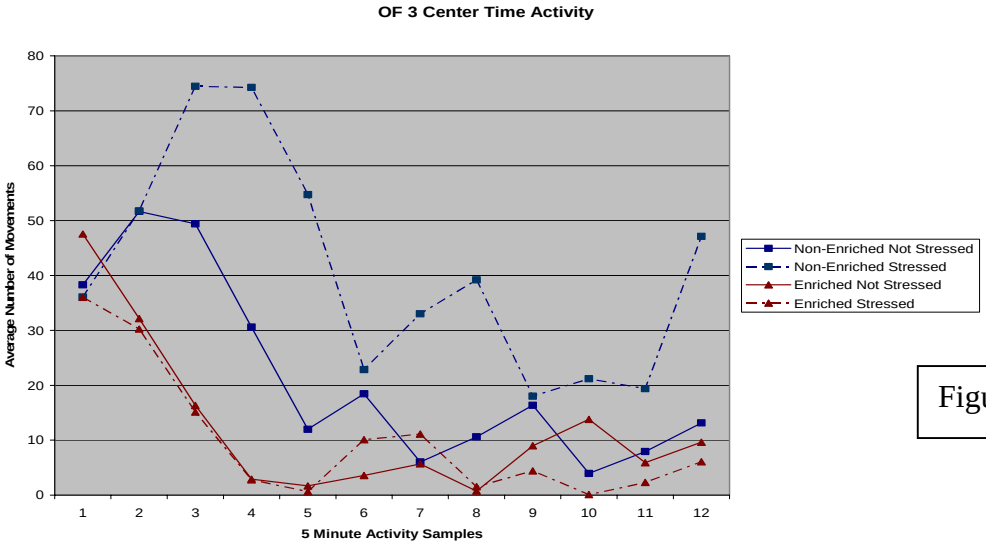


Figure 11c.

Table 8a. Open-Field 4 Horizontal Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	88655.063	1	21703175.111	30.893	.000	.412	1.000
STRESS	38767.785	1	373422.840	.532	.470	.012	.110
ENRCHMT * STRESS	35175.785	1	2238016.000	3.186	.081	.068	.415
Error	1437.674	44	702535.777				

Tests of Within -Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	229867719.118	11	20897065.374	114.052	.000	.722	1.000
TIME * ENRCHMT	15885391.347	11	1444126.486	7.882	.000	.152	1.000
TIME * STRESS	2867185.118	11	260653.193	1.423	.159	.031	.753
TIME * ENRCHMT * STRESS	2019918.792	11	183628.981	1.002	.443	.022	.563
Error(TIME)	88680262.792	484	183223.683				

Table 8b. Open-Field 4 Vertical Activity

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	88655.063	1	88655.063	18.931	.000	.301	.989
STRESS	1437.674	1	1437.674	.307	.582	.007	.084
ENRCHMT * STRESS	1647.007	1	1647.007	.352	.556	.008	.089
Error	206052.750	44	4683.017				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	1344036.868	11	122185.170	81.690	.000	.650	1.000
TIME * ENRCHMT	76861.896	11	6987.445	4.672	.000	.096	1.000
TIME * STRESS	35175.785	11	3197.799	2.138	.017	.046	.927
TIME * ENRCHMT * STRESS	31188.535	11	2835.321	1.896	.038	.041	.886
Error(TIME)	723924.083	484	1495.711				

Table 8c. Open-Field 4 Total Center Time

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRCHMT	38275.662	1	38275.662	19.054	.000	.302	.990
STRESS	23.928	1	23.928	.012	.914	.000	.051
ENRCHMT * STRESS	348.444	1	348.444	.173	.679	.004	.069
Error	88387.627	44	2008.810				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	122948.295	11	11177.118	23.128	.000	.345	1.000
TIME * ENRCHMT	16553.927	11	1504.902	3.114	.000	.066	.991
TIME * STRESS	6031.428	11	548.312	1.135	.332	.025	.630
TIME * ENRCHMT * STRESS	3228.685	11	293.517	.607	.823	.014	.339
Error(TIME)	233901.583	484	483.268				

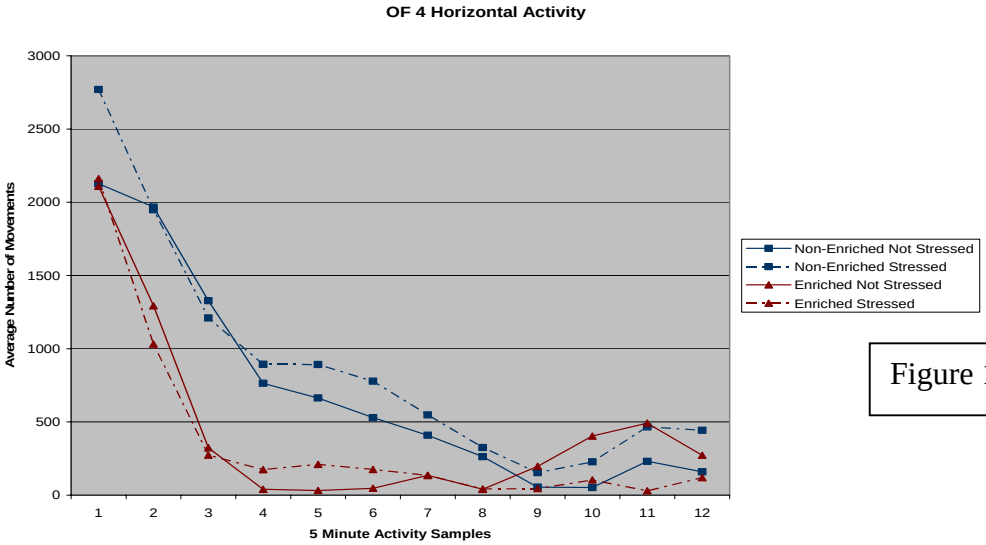


Figure 12a.

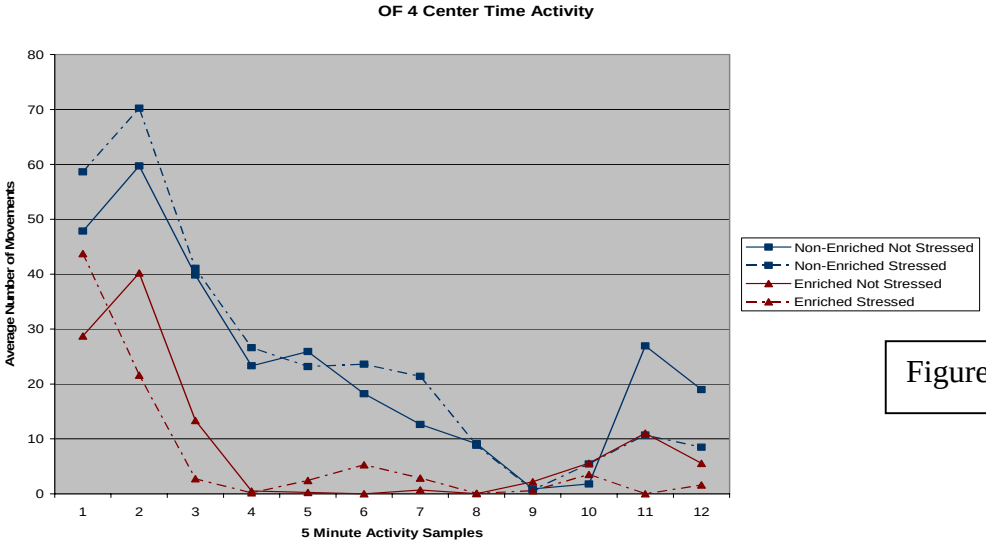


Figure 12b.

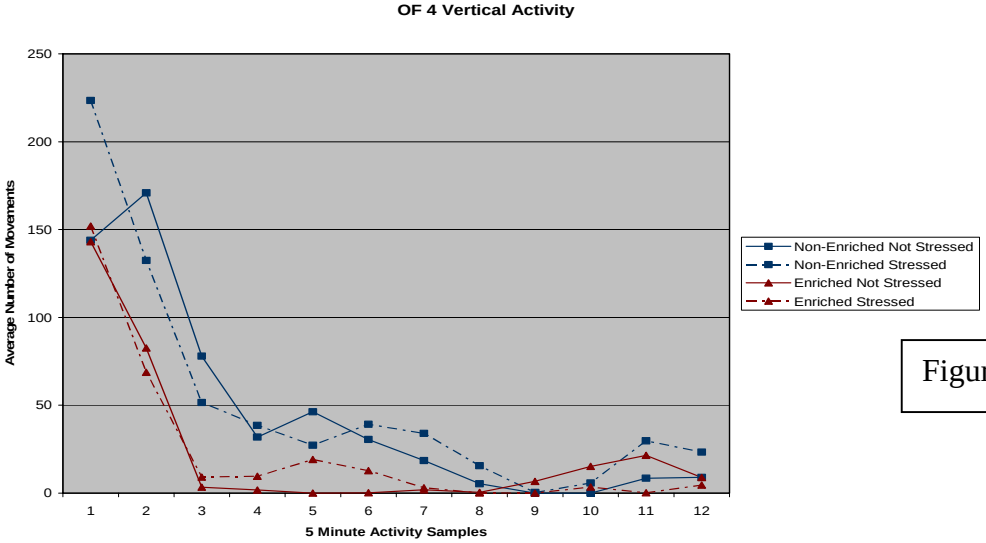


Figure 12c.

Table 9a. Elevated Plus Maze Percent Time in Closed Arms

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Corrected Model	394.022(b)	3	131.341	.775	.514	.050	.203
Intercept	15376.029	1	15376.029	90.715	.000	.673	1.000
ENRICH	368.011	1	368.011	2.171	.148	.047	.302
STRESS	8.464	1	8.464	.050	.824	.001	.055
ENRICH * STRESS	17.547	1	17.547	.104	.749	.002	.061
Error	7457.959	44	169.499				
Total	23228.010	48					
Corrected Total	7851.982	47					

Computed using alpha = .05

R Squared = .050 (Adjusted R Squared = -.015)

Table 9b. Elevated Plus Maze Dependent Variable: Total Crossings

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Corrected Model	2155.229(b)	3	718.410	.170	.916	.011	.079
Intercept	238149.187	1	238149.187	56.490	.000	.562	1.000
ENRICH	1507.521	1	1507.521	.358	.553	.008	.090
STRESS	609.187	1	609.187	.145	.706	.003	.066
ENRICH * STRESS	38.521	1	38.521	.009	.924	.000	.051
Error	185494.583	44	4215.786				
Total	425799.000	48					
Corrected Total	187649.812	47					

Computed using alpha = .05

R Squared = .011 (Adjusted R Squared = -.056)

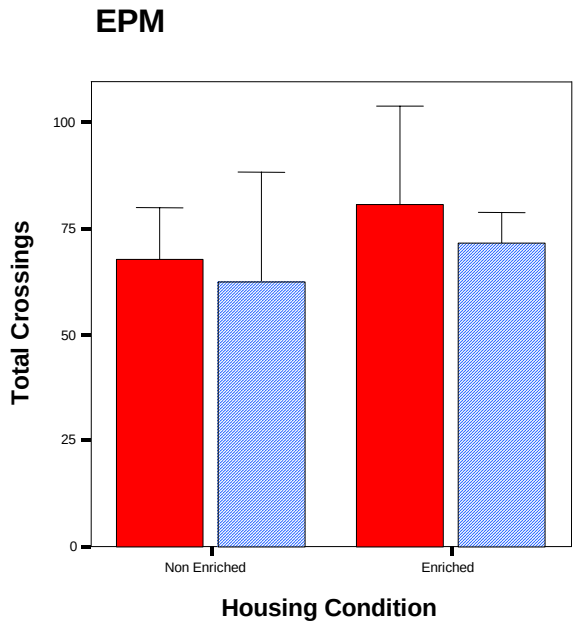


Figure 13a.

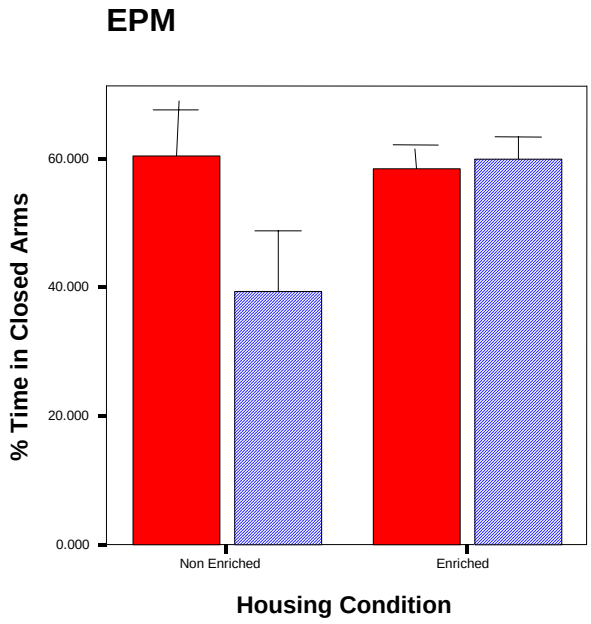


Figure 13b.

Table 10 a. Average Time Spent on Light Side Light/Dark Box

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Corrected Model	6252.539(b)	3	2084.180	2.780	.054	.176	.626
Intercept	134979.363	1	134979.363	180.039	.000	.822	1.000
ENRICHED	3772.866	1	3772.866	5.032	.031	.114	.590
STRESS	1254.909	1	1254.909	1.674	.203	.041	.243
ENRICHED * STRESS	1163.787	1	1163.787	1.552	.220	.038	.229
Error	29239.191	39	749.723				
Total	171777.140	43					
Corrected Total	35491.730	42					

Computed using alpha = .05

R Squared = .176 (Adjusted R Squared = .113)

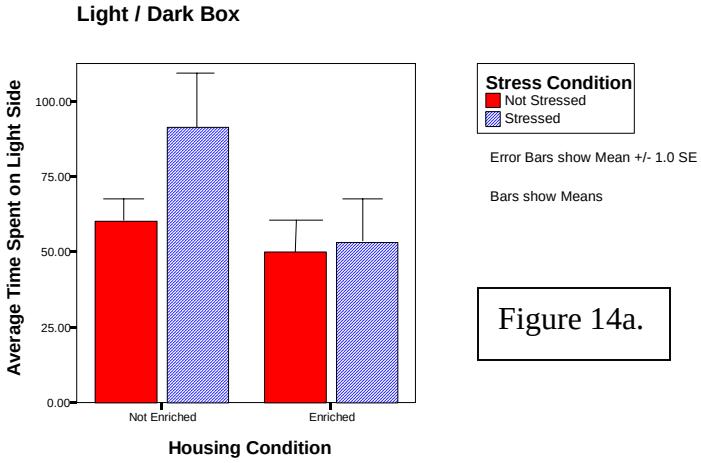


Figure 14a.

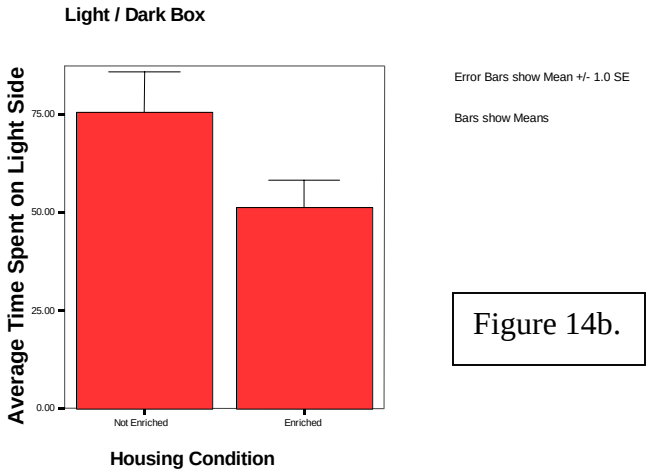


Figure 14b.

Table 11. Plasma Corticosterone

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Corrected Model	167818.032(b)	3	55939.344	1.702	.181	.104	.414
Intercept	11107846.659	1	11107846.659	337.889	.000	.885	1.000
ENRICHED	105614.863	1	105614.863	3.213	.080	.068	.418
STRESS	16692.347	1	16692.347	.508	.480	.011	.107
ENRICHED * STRESS	45510.822	1	45510.822	1.384	.246	.031	.210
Error	1446467.979	44	32874.272				
Total	12722132.670	48					
Corrected Total	1614286.011	47					

Computed using alpha = .05
R Squared = .104 (Adjusted R Squared = .043)

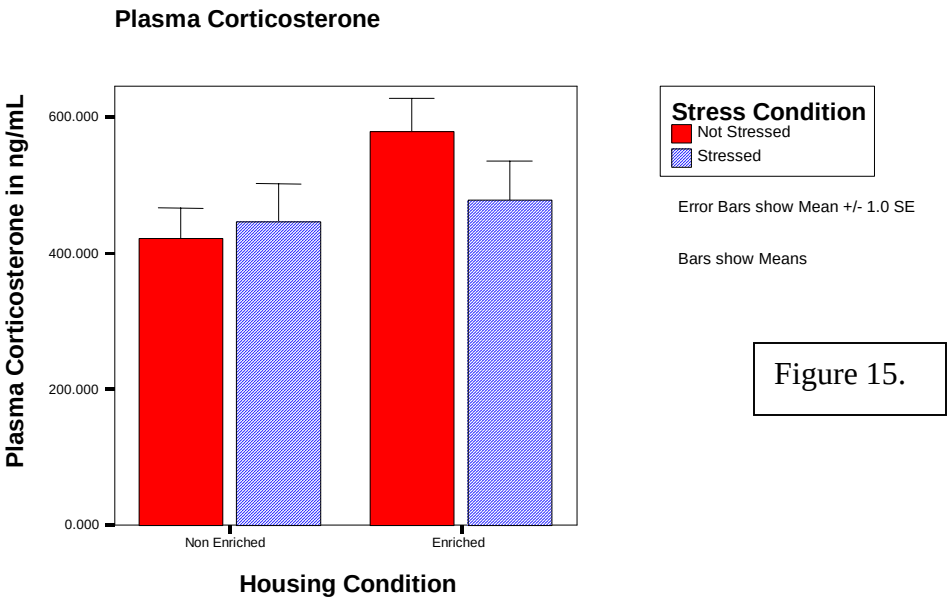


Figure 15.

Table 12a. Phase II Food Consumption

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	974.738	1	974.738	10.435	.002	.185	.885
Error	4297.070	46	93.415				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	9383.238	1	9383.238	369.079	.000	.889	1.000
TIME * ENRICH	310.680	1	310.680	12.220	.001	.210	.928
Error(TIME)	1169.477	46	25.423				

Table 12b. Phase III Food Consumption

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	12.184	1	12.184	.026	.873	.001	.053
STRESS	1945.800	1	1945.800	4.114	.049	.085	.510
ENRICH * STRESS	13609.320	1	13609.320	28.772	.000	.395	.999
Error	20812.198	44	473.004				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	1106.363	1	1106.363	15.193	.000	.257	.968
TIME * ENRICH	169.389	1	169.389	2.326	.134	.050	.320
TIME * STRESS	395.606	1	395.606	5.433	.024	.110	.625
TIME * ENRICH * STRESS	175.771	1	175.771	2.414	.127	.052	.330
Error(TIME)	3204.158	44	72.822				

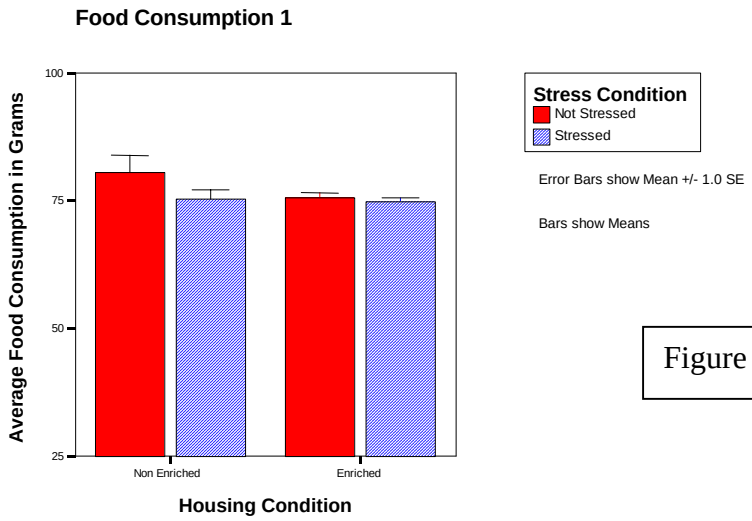


Figure 16a.

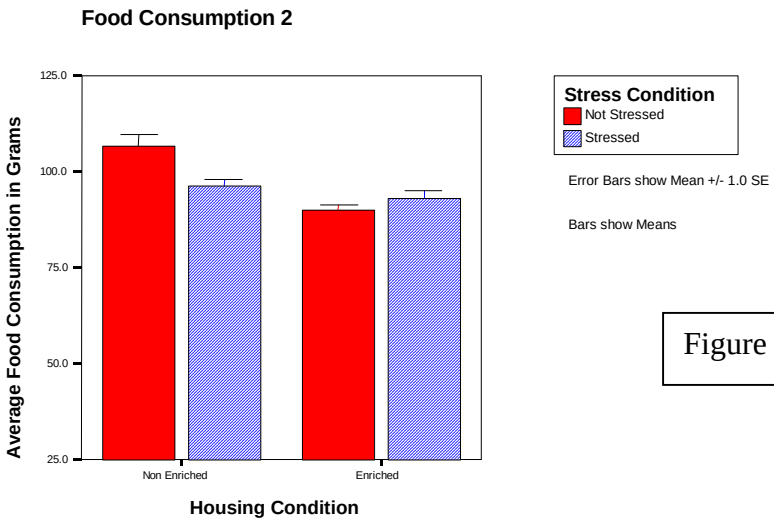


Figure 16b.

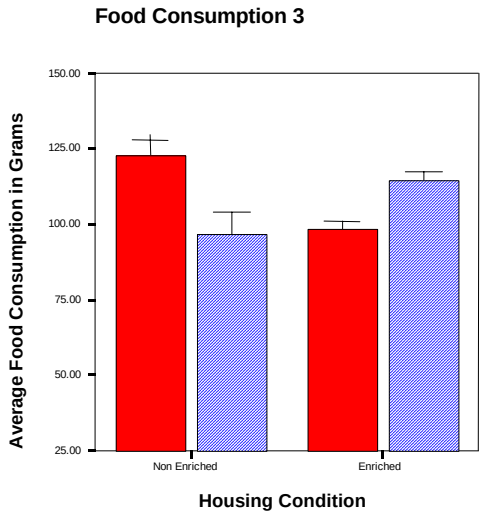


Figure 16c.

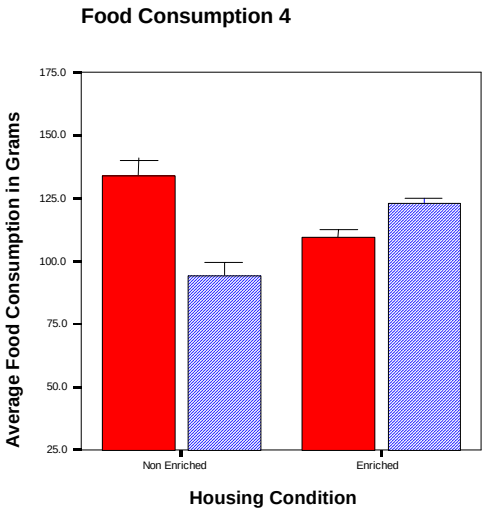


Figure 16d.

Table 13a. Average Food Consumption in Grams Change 1

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	92.408	1	92.408	1.781	.189	.037	.257
Error	2386.433	46	51.879				
Total	283754.160	48					

R Squared = .037 (Adjusted R Squared = .016)

Table 13b. Average Food Consumption in Grams Change 2

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	1193.010	1	1193.010	17.817	.000	.279	.985
Error	3080.115	46	66.959				
Total	449622.130	48					

R Squared = .279 (Adjusted R Squared = .264)

Table 13c. Average Food Consumption in Grams Change 3

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	136.215	1	136.215	.470	.497	.011	.103
STRESS	293.337	1	293.337	1.012	.320	.022	.166
ENRICH *STRESS	5345.897	1	5345.897	18.447	.000	.295	.987
Error	12750.983	44	289.795				
Total	577785.160	48					

R Squared = .312 (Adjusted R Squared = .265)

Table 13d. Average Food Consumption in Grams Change 4

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
ENRICH	45.357	1	45.357	.177	.676	.004	.070
STRESS	2048.069	1	2048.069	7.999	.007	.154	.790
ENRICH * STRESS	8439.194	1	8439.194	32.962	.000	.428	1.000
Error	11265.373	44	256.031				
Total	653625.283	48					

R Squared = .483 (Adjusted R Squared = .448)

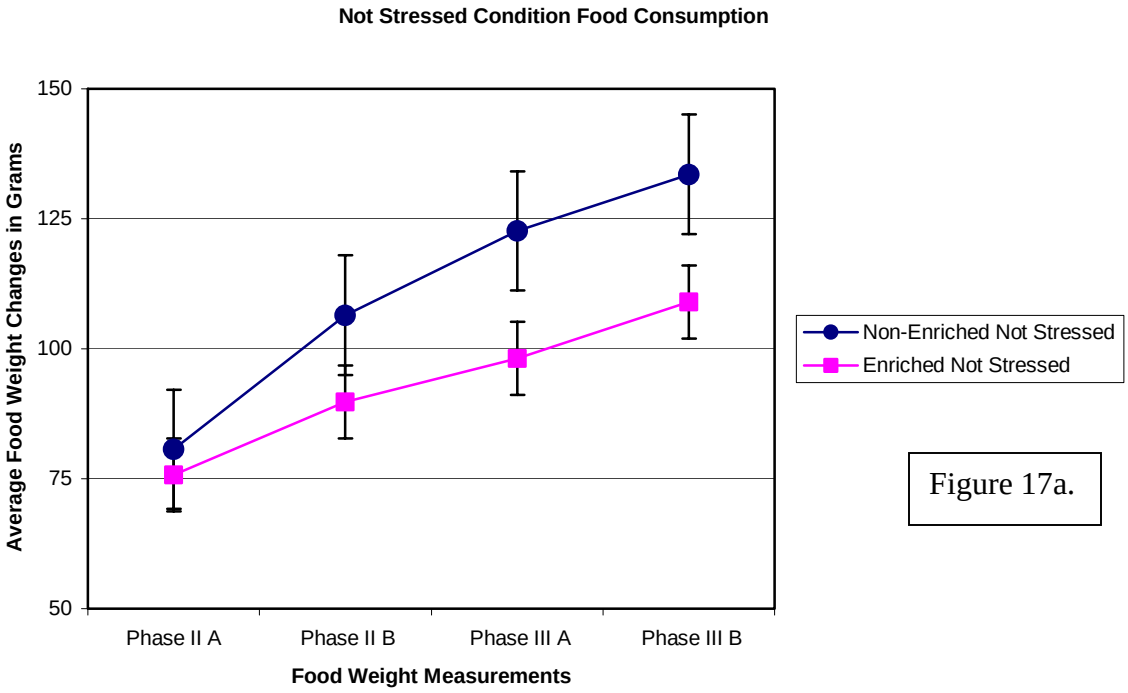


Figure 17a.

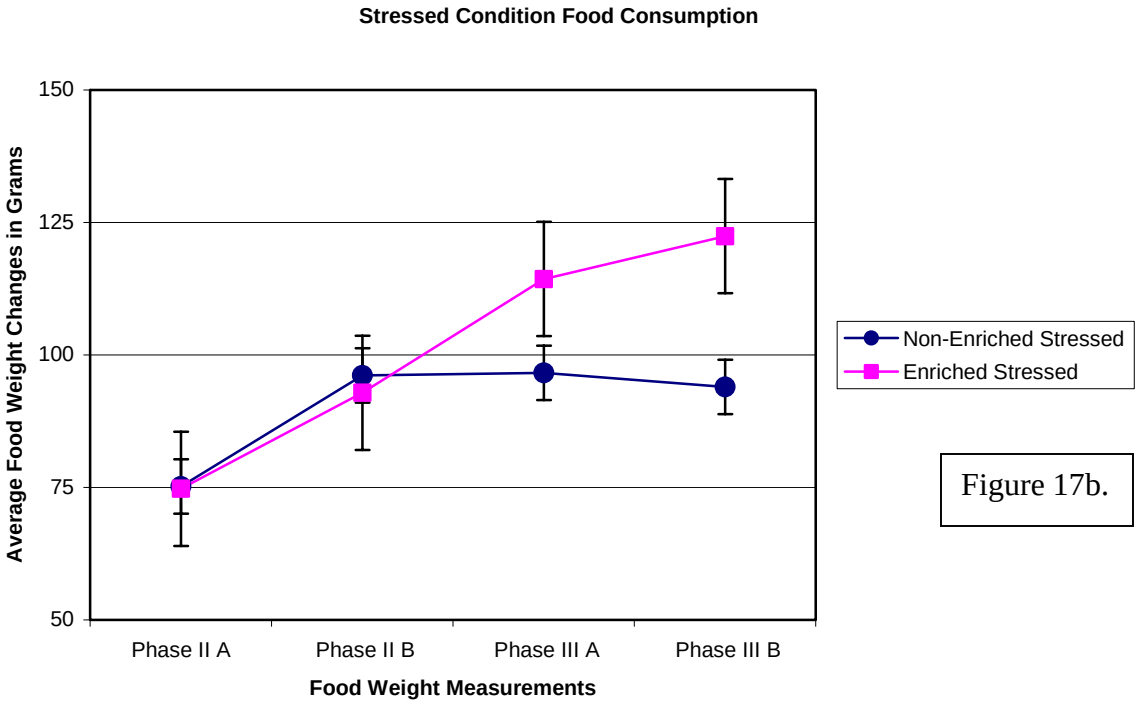


Figure 17b.

Table 14a. Phase I & II

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
HOUSING	732.031	1	732.031	2.258	.140	.047	.313
Error	14915.111	46	324.242				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	292580.648	3	97526.883	2235.657	.000	.980	1.000
TIME * HOUSING	266.464	3	88.821	2.036	.112	.042	.513
Error(TIME)	6020.025	138	43.623				

Table 14b. Phase III

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
HOUSING	5750.694	1	5750.694	7.051	.011	.138	.738
STRESS	965.138	1	965.138	1.183	.283	.026	.186
HOUSING * STRESS	270.000	1	270.000	.331	.568	.007	.087
Error	35885.440	44	815.578				

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
TIME	141891.905	2	70945.952	465.587	.000	.914	1.000
TIME * HOUSING	652.084	2	326.042	2.140	.124	.046	.428
TIME * STRESS	135.565	2	67.783	.445	.642	.010	.120
TIME * HOUSING * STRESS	149.645	2	74.822	.491	.614	.011	.128
Error(TIME)	13409.403	88	152.380				

Computed using alpha = .05

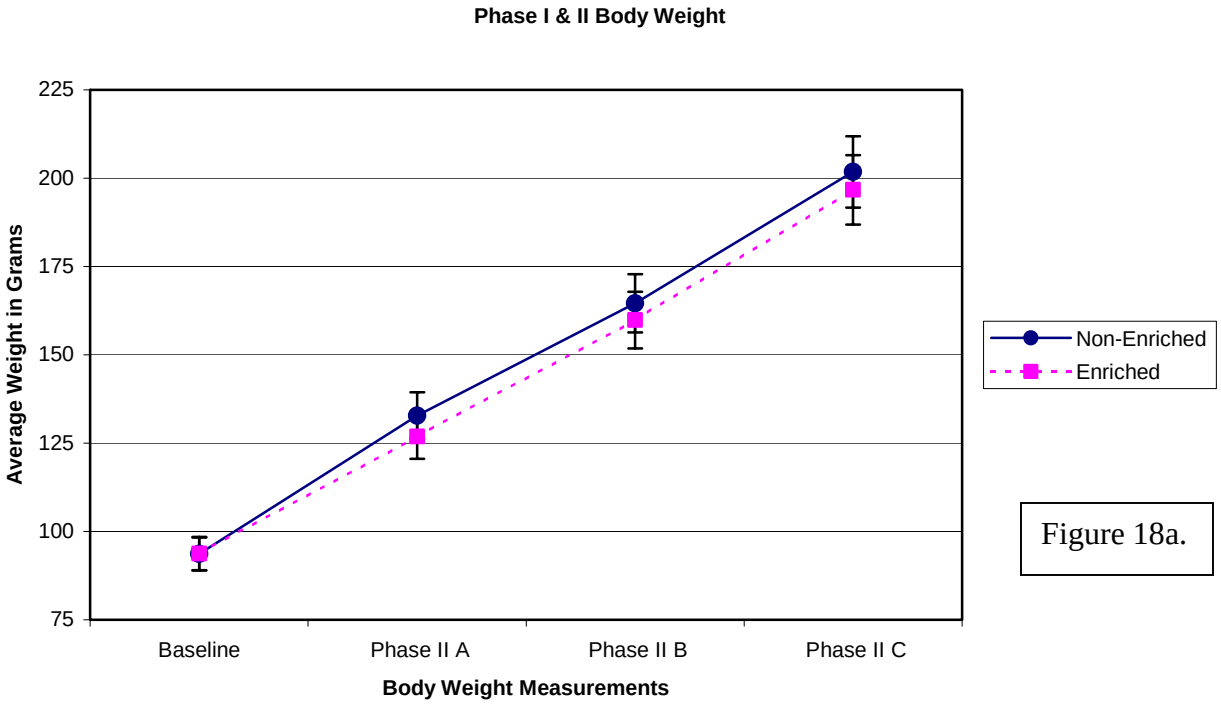
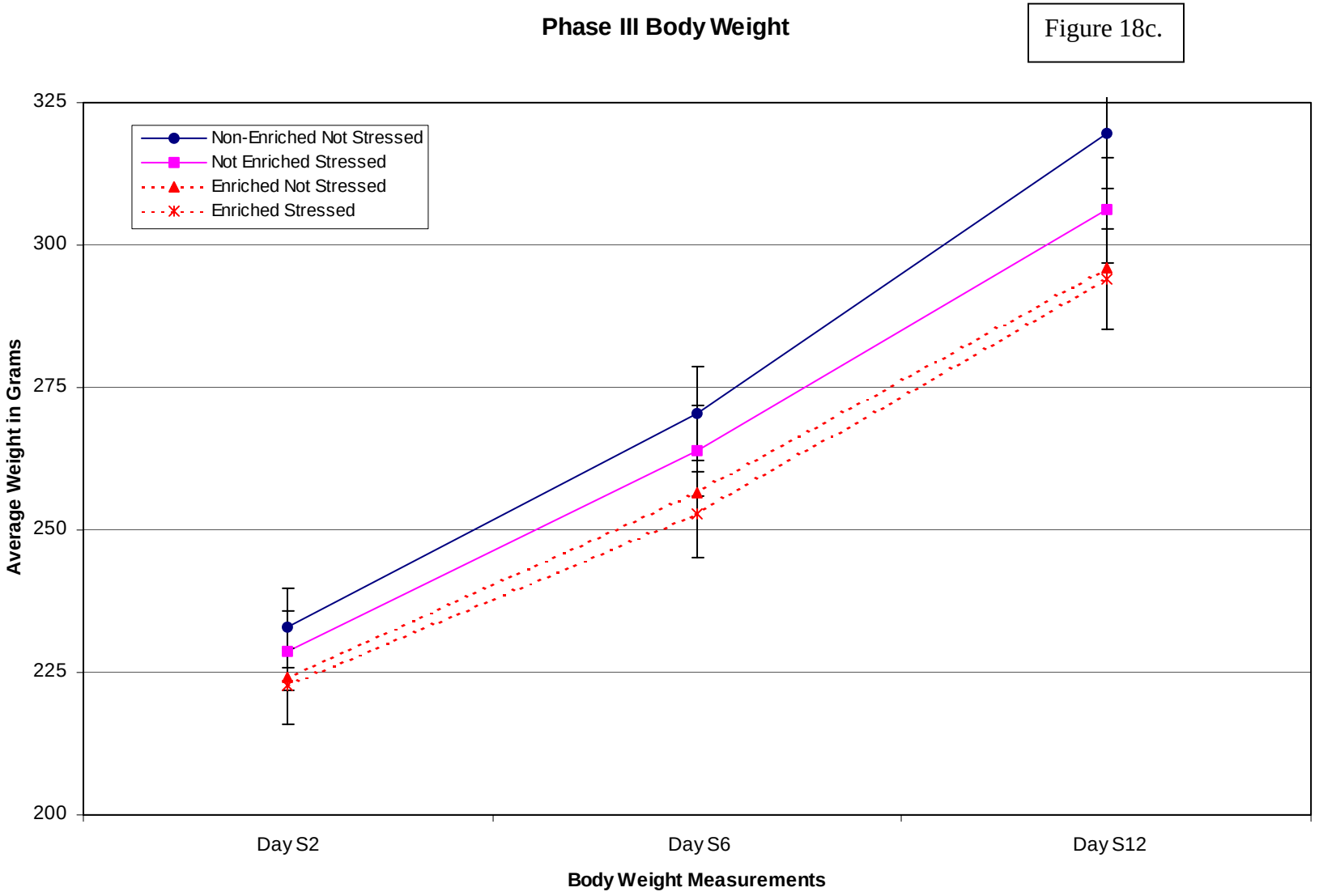


Figure 18a.

Figure 18b.

Phase III Body Weight Results (All conditions)

Figure 18c.



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