LIVE PLANT AND ARTIFICIAL PLANT SETTINGS ARE ABLE TO ALLEVIATE ANXIETY LEVELS IN MICE: AN ELEVATED PLUS-MAZE STUDY

By

SUJEET VERMA

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN HORTICULTURE

WASHINGTON STATE UNIVERSITY
Department of Horticulture and Landscape Architecture

MAY 2009

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of SUJEET VERMA find it satisfactory and recommend that it be accepted.

_____
Virginia I. Lohr, Ph.D., Chair

_____
Raymond M. Quock, Ph.D.

_____
Amit Dhingra, Ph.D.

ACKNOWLEDGMENT

I would like to thank Dr. Virginia I. Lohr for being my adviser, Chair of my Committee, and for her help and support. I would also like to thank Dr. Raymond. M. Quock, Chair, Department of Pharmacology and Toxicology for providing his laboratory and equipments for the experiment and also for his constant constructive comments. I am really grateful to Dr. Amit Dhingra for his support and being in my committee. I am thankful to Donna Sienkiewicz, Vivarium manager, College of Pharmacy for her help. I am really thankful to Eunhee Chung, PhD, Department of Pharmacology and Toxicology for her help during the experiment. I would like to give special thanks to Dr. Rich Alldredge, Department of Statistics, for his suggestions during statistical data analysis.

LIVE PLANT AND ARTIFICIAL PLANT SETTINGS ARE ABLE TO ALLEVIATE ANXIETY LEVELS IN MICE: AN ELEVATED PLUS-MAZE STUDY

Abstract

By Sujeet Verma
Washington State University
May 2009

Chair: Virginia I. Lohr

It has been established that green plant settings around humans can alleviate anxiety, and there is evidence that the response may be partly innate. This experiment was designed to find out if a similar innate response occurs in rodents. Elevated Plus-mazes have been used to measure anxiety levels in mice in pharmacological studies. They consist of 2 open arms and 2 closed arms. A less anxious mouse is expected to explore the open arm more frequently and stay there for longer periods of time. In this experiment, there were two treatment groups and a control. The control had nothing around the maze, the live plant treatment had live plants at the ends of each arm, and the artificial plant treatment used silk plants that resembled the live plants. Number of entries and time spent in open and closed arms was measured and analyzed using Mixed Linear Models Procedure in SAS. Mice spent significantly more time exploring the open arms during the live plant treatment than in the control ($P < 0.001$) or artificial plant treatment ($P < 0.035$). Animals in the artificial plant treatment also spent significantly more time in the open arms than in the control ($P < 0.021$). In addition, the percentage of entries made by live plant treatment mice into the open arms was significantly higher than in the control ($P<0.002$) and in artificial plant treatment ($P<0.007$) as well. However, it was not significantly different from artificial plant treatment mice ($P< 0.199$). In conclusion, this study showed that mice also appear

to respond innately to nature. In this elevated plus-maze study, naïve mice were found to be the least anxious when exposed to the live plant environment and most anxious in the no plant environment. Response in the artificial plant environment was intermediate. While the response was strong, the fact that the differences between live and artificial plants were not always significant indicates that a better design is required in order to provide a good model system for studying this effect.

TABLE OF CONTENTS

LIST OF TABLES

.

LIST OF FIGURES

Dedication

This work is dedicated to my parents, and my elder brother.

CHAPTER ONE

INTRODUCTION

Many people probably sense the connection between nature and humans, but they might not have realized that there is a growing body of research that validates those feelings (Lohr *et al.* 2002, 2006; Tennessen and Cimprich, 1995; Ulrich, 1984; Ulrich & Zimring, 2004). In 1950s, the U. S. Forest Service came under increasing pressure from emerging movements to place values on the uses of the National Forests beyond their value of the wood for lumber (Lohr & Relf, 2000). These movements created an opportunity for researchers from various social disciplines to begin to document the effects of plants, landscapes, and nature on people. Research related to the impacts of landscape scenes on people started to appear in journals (Balling and Falk, 1982; Ulrich, 1979). Environmental psychologists and geographers used this opportunity to begin to study the effect of plants on mental restoration and stress reduction (Ulrich *et al.*, 1981, 1983, 1984, 1986; Kaplan, *et al.*, 1973, 1987, 1988). At the same time, this also opened a window for horticulturists to explore their views and ideas (Flagler, 1995; Lohr & Pearson-Mims, 2000; Lohr *et al.*, 1996). Medical researchers also have explored the field (Cimprich, 1993; Diette, 2003). Beneficial effects of interior and exterior landscapes on human psychology and physiology are now well known (Lohr & Relf, 2000; Ulrich & Zimring, 2004) and there is growing evidence that the response is partly innate (Kaufman & Lohr, 2008; Lohr & Pearson-Mims, 2006; Ulrich, 1993).

Questions arise about how and why these signals from nature are being perceived by the human brain and how this restorative phenomenon takes place. A plethora of emotional and psychological evidence is available that supports the stress reducing effects of nature, but finding

an easy and reliable way to substantiate these previous findings is a major challenge. There are many appropriate limits on the type of research that may be conducted on humans. A model system using animals to probe these responses further could be ideal to advance our understanding in this area of research.

This study examined the possibility of using mice in an elevated plus-maze as a model system for studying innate responses to nature. The elevated plus-maze, which is commonly used in drug trials to measure anxiety levels in mice (Rodgers & Dalvi, 1997; Wall & Messier, 2001), consists of a cross with two open arms and two closed arms. A less anxious mouse is expected to explore the open arms more frequently and stay there for longer periods of time. There were three treatments: a control group in the maze with nothing around it, a live plant treatment group in the maze with a plant at the end of each arm of the maze, and an artificial plant treatment group with a silk plant at the end of each arm of the maze. If part of the human response to nature is innate, then we would expect to see similar responses in other animals.

CHAPTER TWO

REVIEW OF THE LITERATURE

A. Effects of plants on people

Some of the earliest studies of the effects of plants on people simply examined people's landscape preferences. Ulrich (1979) conducted an experiment with students coming out of a final exam. All students were allowed to view either a set of nature slides or urban slides lacking nature and asked to self-assess the level of recovery fostered by the activity. Overall findings suggested that the natural scenes produced higher levels of positive affects and more fear reduction. Balling and Falk (1982) showed scenes of several biomes to children and adults; they found that children and adults liked the savanna scenes, and the savanna scenes were preferred over jungle or desert scenes by younger people.

Research has shown that plants have a calming effect on people. One simple questionnaire-based study was conducted with patients who had gone through a severe accident or illness and found that a window having a nature view was the most preferred by subjects (Verderber, 1986). Lohr and others (1996) found evidence that supported the stress reducing effects of green plants on workers in a windowless environment. In this study, participants' blood pressure and emotions were monitored while completing a simple but stress-inducing computer based task. They found that the group working with plants was less stressed and 12% more productive. This evidence was substantiated when Dijkstra and others (2008) did an experiment in which 77 humans were exposed to a room having either indoor plants or a painting of an urban environment on the wall and measured the subjects' stress and the perceived attractiveness of

their surroundings. Participants exposed to the room with indoor plants were found to be less stressed as compared to the control group.

Studies have shown that plants can affect brain functioning. In 1981, Ulrich had subjects view slides of nature scenes with water and vegetation or slides of urban scenes without vegetation. Although all the slides had similar informational content, those subjects who viewed nature scenes were found to exhibit higher alpha brain waves amplitudes than those who viewed urban scenes. The higher the alpha wave amplitude the more relaxed was the person. Bernadine Cimprich (1993), a cancer nurse, developed an intervention method to reduce mental fatigue in breast cancer patients. She assigned tasks that are known to be mentally restorative to half of the patients, who were to perform the tasks three times a week for ninety days, and at the same time, she tested them on attentional restoration tasks. The task selected by most patients in the intervention group was walking in a garden. Within 90 days, the intervention group was found to have higher total attentional scores than the control group, indicating a significant reduction in mental fatigue and depression. Natural green settings have also been shown to help children suffering from Attention Deficit Disorder to function better (Taylor, *et al.*, 2001). In this study, parents were surveyed regarding their children's attentional functioning with respect to several green settings. It was found that the greener the child's play area was, the less severe their Attention Deficit symptoms were. In addition, Lohr and others (2006) did an experiment to examine the preferences of humans towards various forms (spreading, rounded and conical) of trees. More than 200 participants viewed slide images of spreading, rounded and columnar trees and participants were found to be happiest when viewing the spreading form of trees compared to other forms. Again supporting the idea of landscape preferences and its benefits, Tennessen and Cimprich (1995) did an experiment with 72 undergraduate students in order to find out

whether university dormitory rooms having natural views through their windows helped students in scoring better on exams. It was found that students with a view of nature out of the dormitory window scored better than those with a view of hardscape, such as sidewalks or parking lots.

B. Evolutionary history and theoretical aspect

Humans started to adopt themselves according to the environment for survival. During the process of mental development, the human brain encountered various setting that appeared first time in its life. The theory of the evolution of natural perception explains the idea of the development of specific neuronal areas (Joye *et al.*, 2007). It also explains how various neuronal areas became specialized to differentiate between natural and non-natural things under the evolutionary pressure of survival related challenges that were available to our ancestors.

Researchers have proposed different reasons for human responses to nature. According to Roger Ulrich (1993), responses towards environmental settings are not cognitive phenomena that involve conscious thought. Instead these responses are mediated by quick, automatic and unconscious processes that lead to liking or nonliking preferences for a particular environment. These are found to be adaptive responses and deeply rooted inside human evolutionary history. For example, when early humans came across risky conditions like turbulent water or a predator, a negative response was triggered leading to an avoidance behavior. Ulrich (1993) postulated that the restorative property of nature is initiated immediately and unconsciously leading to physiological and behavioral responses. Ulrich (1993) also suggested that these quick-onset emotional reactions would trigger adaptive processes and motivate avoidance behavior with little cognitive activity required. In addition to that he suggested that these adaptive processes should happen within minutes depending upon the intensity of the stress. Ulrich (1993) emphasized that attention or interest would be a key element for both restorative and stress responses towards natural setting containing risk or threat. On the contrary, if a setting offered opportunities for survival and reproduction, a positive response could have streamed into the brain and consequently liking and explorative behavior would follow. According to this

psychoevolutionary framework (S. Kaplan, 1987, 1988; Ulrich, 1983), if these instant reactions had some inherited component, then no precious time and energy had to be spent learning what kind of environment is beneficial and harmful and consequently, the chances of survival would increase.

The phenomena of aesthetic preferences for a landscape were further explained by Jay Appleton (1975). He was the first person to propose the "prospect-refuge theory." According to him, prospect and refuge are the two environmental qualities that correlate with human's landscape preferences (Appleton, 1975). The word "prospect" refers to a setting that facilitates our brain to obtain more information about the environment. At the same time, "refuge" sends a signal of protection and shelter. In addition, according to the Kaplans' model (R. Kaplan & Kaplan, 1989; S. Kaplan, 1987, 1988), there are two types of activity towards the environment, explorative and assessment. An individual can actively explore a setting or try to understand the environment. According to Kaplan and Kaplan (1989), the structural properties that facilitate involvement in a particular setting, are "complexity" and "mystery". Stephen Kaplan (1989) defines complexity as a measure for "how much there is to look at" and mystery refers to settings whose structural features suggest that "there could be more information if the setting is penetrated deeper".

There are several other theories about the phenomenon of mental responses to nature in humans. One proposes that an exposure to nature has the potential to foster physiological well being and restoration after exposure to stress from daily urban living (Ulrich & Parsons, 1990). Fredrick Law Olmsted (1865), a famous landscape planner and architect from the USA, discussed the stress associated with urban cities and came up with the argument that viewing nature is potentially able to alleviate stress. He created many city parks such as Central Park in

New York City that had an influence in shaping cities for the better. Many social scientists have proposed that an encounter with the most unthreatening nature settings will have a stress reducing effect, but at the same time many urban environments will hinder convalesance (Ulrich and Simons, 1986).

A famous theory called the arousal theory (Berlyne, 1971) states that a setting having arousal properties, such as movement and complexity, that are low would produce a greater healing effect than settings with greater arousal properties. According to Wohlwill (1976), natural settings have lower complexity levels and arousal properties than urban environments and also have comparatively more restorative properties. Another important aspect that was speculated by Wohlwill (1983) was that any natural content can be easily processed by the brain, because our brain and nervous system evolved in natural environments and urban settings require more adaptation processes. At the same time, if an individual is stressed, the demands placed by such adaptation processing might hinder the process of healing (Stainbrook, 1968).

Kaplan and Kaplan (1989) proposed a theory about attentional restoration to explain human responses to nature. They speculated that restorative influences are cognitively-based and are aroused by the attention holding properties of settings such as the configurations of the landscape. They claimed that there is a preference matrix embedded deep within the brain that cognitively perceives the presence or absence of specific information in a particular environment (Kaplan & Kaplan, 1989). The Kaplans' theory was supported by Olmsted's (1865) earlier argument that nature held attention without any mental effort and that was the reason for the restoration property of nature. This theory was also supported by Cimprich's work (1993) with breast cancer patients (described above).

C. Neurology and endocrinology of stress

Every human being follows a biological clock throughout his/her life unknowingly. It is the very first light of dawn that triggers the biological clock in all mammals (University of Virginia, 2008). It has been suggested that the first light which strikes the retina produces a sensory signal that moves through the optic nerves and delivers a message to the brain center. The brain center then fosters the production of regulatory hormones that move into the blood stream, preparing the body and mind to anticipate the environment and behavior (Doyle and Menaker, 2007).

Stress physiology in mammals is affected by two different but cross-talking systems of the brain: the sympathetic-adrenomedullary system and the hypothalamic-pituitary-adrenocortical system (Gunnar and Quevedo, 2007). The sympathetic-adrenomedullary system is a component of the autonomic nervous system releasing adrenaline, whereas the hypothalamic-pituitary-adrenocortical system produces glucocorticoids such as corticosterone in rodents and cortisol in humans (de Kloet *et al.*, 1996, 1991). Hypothalamic-pituitary-adrenocortical products are able to cross the blood brain barrier and have major effects on the brain. Adrenaline produced quickly by the sympathetic-adrenomedullary system plays a major role in the flight/fight phenomena, and unlike adrenaline, glucocorticoid production takes longer and many of its affects on the mammalian body and brain occur through changes at the genetic level. Consequently, glucocorticoids have slower effects than sympathetic-adrenomedullary hormones, but they continue for longer periods of time (de Kloet *et al.*, 1996, 1991). Now, looking precisely to the overall picture of stress responses, it seems that both the sympathetic-adrenomedullary and the hypothalamic-pituitary-adrenocortical systems converge in the hypothalamus, which connects autonomic and endocrine function with behavior (Palkovits, 1987).

Sympathetic-adrenomedullary system: The chromaffin cells of the adrenal medulla, which are considered as parts of sympathetic nervous system, secrete catecholamines (stress hormones similar to adrenaline and nor-adrenaline) when stimulated (Gunnar and Quevedo, 2007). These hormones play a significant role in the fight/flight reaction. Under the condition of any threat and psychosocial stressors, catecholamines are secreted and general physiological activities, such as cardiac output, heart beats, and pupil dilation, are enhanced. The overall goal of the sympathetic-adrenomedullary system is to increase vigilance and activate processes that stimulate the hypothalamic-pituitary-adrenocortical system (Gunnar & Quevedo, 2007). Risk assessment behavior of mice in an elevated plus-maze can be considered as a pragmatic example of sympathetic-adrenomedullary and hypothalamic-pituitary-adrenocortical system interaction, where exploratory avoidance behavior is fostered in anxious mice whereas less anxious mice would explore maze.

Hypothalamic-Pituitary-Adrenocortical system: The main function of the hypothalamic-pituitary-adrenocortical system is the secretion of adrenocorticotropin hormone stimulated by corticotrophin-releasing hormone from anterior pituitary (Charmandari *et al.*, 2005). As soon as the paraventricular nuclei of hypothalamus receive signals from the hippocampus, they release corticotrophin releasing hormone and arginine-vasopressin. Corticotrophin releasing hormone and arginine-vasopressin then travel to the anterior pituitary where they induce the secretion of adrenocorticotrophine. Adrenocorticotrophine then interacts with the adrenal gland and stimulates the production and release of glucocorticoids into the systemic circulation and brain (Charmandari *et al.*, 2005). Here glucocorticoids interact and activate receptors that finally regulate the transcription of genes with glucocorticoid–responsive regions. Since, glucocorticoid activity involves changes at the genetic level, it takes hours to show a response and continuous

production of glucocorticoids may alter physiology and behavior over time (Sapolsky *et al.*, 2000).

The effect of glucocorticoids depends upon the nature of the receptor with which they bind (Gunnar and Quevedo, 2007). Moreover, there are two types of receptors: mineralocorticoid receptors and glucocorticoid receptors. The binding nature of mineralocorticoid receptors and glucocorticoid receptors depends upon the concentration of glucocorticoids relative to the brain. Outside the brain, glucocorticoid receptors are the main receptors that bind with glucocorticoids, because an enzyme called 11 beta-hydroxysteroid dehydrogenase inhibits glucocorticoids from binding to the mineralocorticoid receptors. On the contrary, inside the brain where 11 beta-hydroxysteroid dehydrogenase is less expressed, glucocorticoids bind with both mineralocorticoid receptors and glucocorticoid receptors, but mainly with mineralocorticoid receptors (Gunnar and Quevedo, 2007). Since, glucocorticoids have a higher affinity for mineralocorticoid receptors at the basal level; they can play a major role in the hypothalamic-pituitary-adrenocortical system during stress responses.

Glucocorticoid receptors are involved in most stress related effects; however mineralocorticoid receptors mediate most of basic physiological effects such as neurotransmission, circadian cycle and blood-pressure (Sapolsky *et al.*, 1997). Even at the basal stress level, glucocorticoids can bind to most of the mineralocorticoid receptors inside the brain and alter basic physiological functioning. Therefore, elevated level of glucocorticoids can affect both psychology and physiology. Although glucocorticoid receptors are involved in acute stress responses, they are also involved in negative feedback inhibition leading to the termination of hypothalamic-pituitary-adrenocortical stress responses. It has been suggested that the suppressive effects of glucocorticoid receptors are necessary to reverse stress responses and regain cellular

homeostasis. However, the benefits of this suppressive effect are overturned when the stressor is prolonged (Sapolsky *et al.,* 1997). Therefore, it can be concluded that prolonged exposure to the suppressive effect of glucocorticoids can have hazardous effects on physical and mental health.

Role of corticotrophin hormone: According to researchers (Joye, 2007; Parson 1991), the liking and nonliking preferences of humans to the environment have an important connection with subcortical areas of the brain, especially with the amygdyla. Since these structures are involved in modulating stress hormones, it might explain the phenomenon of an autonomic stress response towards various settings. It has been found that the limbic brain system manipulates the sympathetic-adrenomedullary and hypothalamic-pituitary-adrenocortical systems, which also involve the amagdyla, hippocampus and orbital prefrontal cortex. These components permit psychological stressors to activate stress responses. Corticotropin releasing hormone is found to be involved in cortico-limbic pathways that mediate fear and anxiety related behavior. Corticotropin releasing hormones also play a pivotal role in activating pathways of both hypothalamic-pituitary-adrenocortical and sympathetic-adrenomedullary responses. The amygdalar-corticotropin releasing hormones are being studied extensively for their critical role in modulating stress reactions (de Kloet *et al.*, 1996, 1991; Heinrichs *et al.*, 1995). In rodents corticotropin releasing hormones infused into the locus coeruleus increase anxiety related behavior, and after being exposed to psychological stressors, neurons in the locus coeruleus are sensitized to corticotropin releasing hormone (Butler *et al.*, 1990) and consequently affects behavior. There are two types of corticotropin releasing hormone receptors like glucocorticoids; corticotropin releasing hormone-1 and corticotropin releasing hormone-2. These two receptors act antagonistically to each other. For example, corticotrophin releasing hormone-1 receptors are common in cortico-limbic-pathways that mediate fear and anxiety related behaviors, whereas

corticotrophin releasing hormone-2 receptors are mostly found in sub-cortical brain regions and mediate most of stress effects on body functions like blood pressure, heart beats, body temperature etc. (Sanchez *et al.*, 2000).

Overall, when mammals come under an environment that has stress provoking components, both hypothalamic-pituitary-adrenocortical and sympathetic-adrenomedullary systems start acting according to the message received to the brain center (Sapolsky *et al.*, 1997). Meanwhile, stress hormones are released within the body and in order to cope with a stressful external environment, unusual physiological phenomenon build up within the body, giving a feeling of sustainability against the threat for a while. But now, it is clear that stress hormones have deleterious effects on brain related activities and also on basic physiological activities such as blood pressure and heart beats if continued for long periods of time (Sapolsky *et al.*, 1997).

D. Mice as an animal model for humans

From many years, mice have been used as an animal model for scaling the impacts of drugs and diseases on humans (Spencer, 2009; Elfline et al., 2004). Mice are used in biomedical and neurological research as models for humans in order to understand the human body and behavior, determine the effects of diseases, and develop treatments for diseases. In pharmaceutical research, before human clinical trials, toxicity and fatality (lethal dose) are tested on mice. Mice have also been used to study behavioral effects of anxiolytic compounds like benzodiazepine and $GABA_A$ receptor agonists (Elfline et al., 2004).

Mice and humans both have about 30,000 genes and both share 99% of them (Spencer, 2009). Mouse-human genome comparisons led to the discovery of about 1200 new genes in humans (Russell, 2002). Ninety percent of the genes associated with disease are found to be identical between the human and the mouse, supporting the use of mice as model organisms for humans (Russell, 2002). Further analysis of mouse and human genomes reveled that man and mouse are cousins evolved from *Eomaia scansoria*, which was the earliest known representative of the *Eutheria* lineage which gave rise to all placental mammals (Russell, 2002). A striking similarity of a possible connection between genetic regulation of an altered circadian cycle and behavioral disorders in mice and humans suggested that the mouse can be used for behavioral study for humans (Wager-Smith & Kay, 2000).

Human response to nature is often initiated by seeing plants. Since, we are proposing to study mouse response to plants; it is important to compare what humans and mice actually see. Humans have trichromatic vision, consisting of short wavelength blue-cones, middle wavelength green-cones and long wavelength red-cones. Rodents have dichromatic vision (Radlwimmer,

1998). They have short wavelength blue-cones, middle wavelength green-cones. The blue-cone wavelength peak sensitivity is at 359nm, and the green cone wavelength peak sensitivity is around 510nm. Humans can see colors within 400nm to 700nm wavelength range. About 88% of a rodent's cones are the middle green type and about 12% are of short blue cones. Rodents don't have many cones: 99% of the rodent's retina consists of rods, which sense only light and dark and only 1% consists of cones (LaVail, 1976), compared to a human's 5% of cones (Hecht, 1987). Rodents are colorblind (Crawford *et al.,* 1990) i.e. they can't differentiate among all colors. However, they can easily differentiate blue from green. Recent studies (Jacob *et al.* 1991, 2001) proved that rodents can easily differentiate between visible and ultraviolet light and if provided with training they can even discriminate dichromatic color wavelengths. Rodent's color perception is not as clear as that of human's; brightness is more important than color cues for them (Jacob *et al.* 1991, 2001). In summary, rodents are physically capable of distinguishing between ultraviolet, blue and green light (Nelson and Marler 1990).

E. Mouse behavior on the Elevated Plus-maze

Various kinds of mazes have been used for the estimation of several psychological and physiological effects in rodents. Scientists have been using elevated plus-mazes for psychopharmacological and behavioral studies to measure anxiety and behavior in rodents for a long time. One early maze was the Y-maze. Montgomery (1955) characterized the Y-maze as having a conflicting novel environment nature, because of the fear generating property of its open arm and subsequent protected feature of the closed arm. Finally, Montgomery (1995) concluded that a novel environment was able to stimulate both a fear drive and an exploratory drive in rodents. However, Weiss at al. (1998) suggested that explorative behavior could be independent of the fear drive in a novel environment; instead it could be because of the reduced fear and enhanced explorative effect of the novel environment. The dynamic relationships among novelty, fear, exploration and motivation in mazes had been major topics for debate (Weiss et al., 1998). Later on, Handley and Mithani (1984) modified the maze and showed that a maze of a plus shape with two opposing perpendicular open and closed arms was potentially able to measure anxiolytic effects in rodents by simply measuring the ratio of open to total arm entries. In addition, Pellow et al. (1985) added the ratio of time spent in open arms to the total time spent on the maze and also the ratio of each arm entry to the total arm entries as major parameters for physiological measurements.

It has been observed that rodents in mazes spend a lot of their time staying still or moving from one part of the maze to another (Antoniou et al, 2004). There are other common types of behavioral responses, such as rearing (body inclined vertically with hindpaws on the floor of the maze and forepaws on the wall of the cage), sniffing (sniffing parts of the wall and floor), grooming (washing the face or any other part with the forepaws), and stretching (stretching the

middle part of the body while the forepaws and hindpaws stay in the same quadrants). Moreover, rodents exhibit a very common behavior in the maze called “risk assessment” (Rodgers et al., 1997). The term “risk assessment” usually refers to specific stretching postures and peering over the side of the open arms. More specifically, during the risk assessment period rodents would assess an open arm from a safer place in the maze. A rodent might do this by poking its head out onto an open arm while standing at the edge of a closed arm. In another way, we can say that rodents might engage in this kind of risk assessment while standing securely in the center of the maze. Behavioral scientists also tend to refer to looking over the edge in the open arms as risk assessment. These include head dipping (leaning over the edge of the open arm) and stretching (stretching forward and retracting back without moving the feet). These actions can take place in the closed arm (protected) and open arm (unprotected). Together with several other measures of hesitancy and inactivity, these behaviors are collectively called as risk assessment behavior (Dawson et al., 1995). Pharmacology, anxiolytic compounds tend to decrease risk assessment behavior and increase unprotected activity. In addition to that there is another kind of behavior called ‘escape behavior’. Sometimes rodents exhibit extreme behaviors like purposefully jumping to the floor from the open arm. Since the rodent is trying to escape, this is referred as ‘escape behavior’ (Kalynchuk, et al., 1997).

F. Behavioral Profiling

The very first step in any kind of animal study is to understand the behavior of animals with the apparatus being used in the experiment. In our study we used the elevated plus-maze for the measurement of anxiety levels in mice. In this section of my thesis, I will discuss general behavior of mice on the maze and its interpretation in relation with physiology and psychology. Basically, the behavior of mice on maze can be divided into two parts: (1) explorative behavior and (2) non-explorative behavior. Parameters such as time spent in open arms, open arm entries, time spent in closed arms, closed arm entries, and time spent in the center have been focused as significant features of exploration (Rodgers et al., 1997). However, significantly identifiable movements such as rearing, head-dipping, stretching postures, grooming, freezing and returning to closed arms are considered as non-explorative behavior of rodents on the maze (Rodgers et al., 1997). Table 1 below illustrates major behavioral elements and their interpretations in relation to the overall behavior of rodents. In addition to the above mentioned behavioral elements, genetic variation in mice strains (Hinojosa, et al., 2006; Izidio, et al., 2005) and thigmotactic cues are major points of concern during behavioral profiling of rodents (Rodgers et al., 1997). It has been suggested that the presence of walls in the closed arm could be the primary reason for open arm avoidance, not height of the maze above the floor (Rodgers et al., 1997).

Table 1 Various behavioral elements in rodents and their interpretations (Table based on Rodgers et al., 1997, pp-294)

Behavior	Interpretation	Behavioral Elements
Explorative	Anxiety	Open arm entries
		Open and closed arm time
	Locomotion	Total arm entries,
		Closed arm entries
		Center and closed time
	Vertical activity	Rearing
	Exploration	Head dipping
		Stretched attend postures
Non-Explorative	Risk assessment	Stretched postures
		Sniffing
	Decision making	Closed arm returns,
		Grooming

G. Research hypothesis

This experiment was designed with an objective to prepare an animal stress model in order to scale the impacts of natural environments on humans. The main hypothesis of this experiment was to determine if the calming effect of nature, which appears to be wired in the human brain, is also wired in the brains of other animals and at the same time to determine if mice could be used as an animal model.

CHAPTER THREE

MATERIALS AND METHODS

Mice

Sixty male NIH-01 mice (Strain 01S50 – Cr), weighing 23-28 g (5 weeks old), were purchased from National Cancer Institute at Frederick, Maryland, USA and used in this experiment. The experimental procedure was reviewed and approved by Institutional Animal Care and Use Committee of Washington State University, Pullman (protocol number 03759-001) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Academy Press. Mice were housed four per cage (made of acrylic) and provided with food and water *ad libitum*. All animals were housed at 20 - 22.2 degrees Celsius temperature, 28 to 32 percent relative humidity and a 12-hour dark and light circadian cycle. No mouse was treated with any drug. A complete non-invasive method was employed for this experiment.

Elevated plus maze

The elevated plus maze used in this experiment was constructed with black Plexiglas. Each arm was 30 cm long and 5.5 cm wide, and the open square in the center was 10 cm by 10 cm (Figure 1). Each closed arm was enclosed by 16 cm high black walls. The arms were cross-fixed at an angle of 90^0 to one another, and there was no roof at the top of the closed arm. These arms were mounted on legs 24 cm in height.

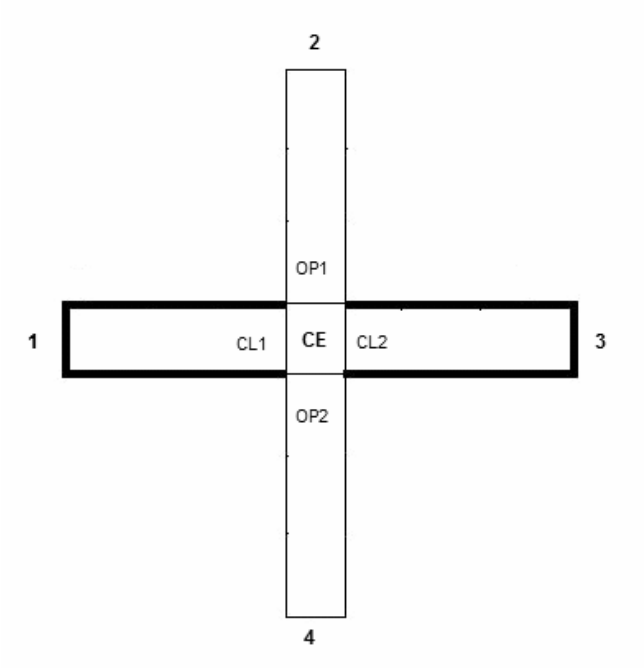


Figure 1: Diagram of an elevated plus maze. Dark lines indicate the walls of the closed arms and light lines represent the border of the open arms. OP1and OP2 are used to represent the two divisions of the open arms, and CL1 and CL2 are used to represent the two divisions of the closed arms. The arms are separated by a center area called CE. Numbers at the ends represent plant positioning.

A video recorder was placed above the maze for data collection. It was mounted at an angle of approximately 80 degrees in order to have a full view of a mouse in all parts of the maze. For precise identification of each mouse, a card having subject number and treatment symbol was placed within the camera view.

Experimental design and treatments

This experiment was designed as a randomized complete block design with three treatments, five replications, and four mice as the experimental unit. There were two treatment groups and one

control group in this experiment. The control group was tested in the elevated plus maze with nothing around it (Figure 2). The first treatment group, called *live plant treatment*, was tested in the maze with one live plant placed at the end of each arm; one green plant and one plant with red coloring were used for the open arms and different species of green and red plants were used for the closed arms (Table 1.1; Figure 3). The plants were placed at the ends of the arms, so that the open arms would retain exposure for the mouse, but so that the canopy might be visible over the closed arms at the ends of the arms. The plants were placed in such a way that mice could not reach the plants placed at the end of arms. The second treatment group, called *artificial plant treatment* (Figure 4), used artificial plants that were similar in size and color to the live plants, and the plants were placed the same way as those in the live plant treatment. Four mice were selected as the experimental treatment to reduce the time needed to switch plants between treatments and to reduce the chance for plant damage in the process.

The experimental design was not selected to test mouse response to color, but a modification was made to the design during the experiment. During the first two replications, it appeared that the mice were more likely to enter some arms than others. We speculated that differences within the lab, or differences based on plant color, or chance might be causing the perceived response. As a result, the design was modified during the experiment by switching the plants' positions within the sub-arms to reverse the locations of the green and red plants. This was done after the second replication was completed.

Table 1.1 Scientific name, common name and foliage color of plants used in the experiment

Scientific name	Common name	Foliage color
Chamaedorea elegans	Neanthe bella palm	Green
Codiaeum variegatum	Croton	Red, green, and yellow
Nephrolepis exaltata	Boston fern	Green
Solenostemon scutellarioides	Coleus	Red

Figure 2 Picture of the elevated plus maze for the control treatment

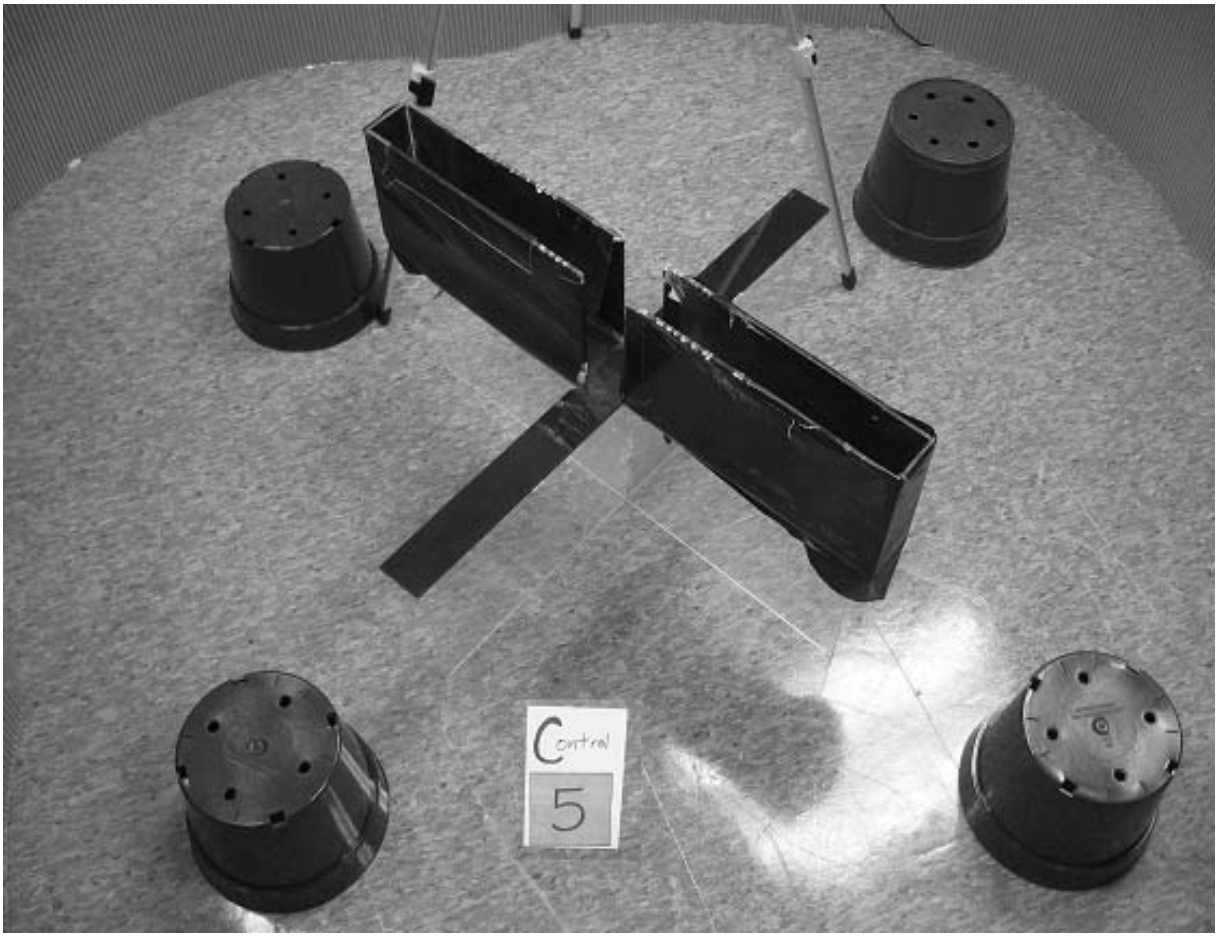


Figure 3 Picture of the elevated plus maze for the live plant treatment.

Figure 4 Picture of the elevated plus maze for the artificial plant treatment setting.

Procedures and data collection

The mice were moved to the room where the experiment was conducted approximately 120 minutes before beginning the experiment for acclimation. The maze was prepared for the randomly assigned treatment. The video recording was started, and then one animal was removed from its holding box and carefully placed in the center of the maze facing towards an open arm. It was allowed to explore for 5 minutes and recorded. All mice were exposed to the maze only once in this experiment. If an animal fell off of the maze before the five minutes had passed, it was picked up and placed immediately back in the center of the maze (Alicia et al. 2007); recording continued to attain a full 5-minute treatment period. The mouse was then removed from the maze and placed in a different holding box. The maze was wiped clean with a dry paper towel. Mice were sequentially placed in the maze following the same procedures. After every four mice had been tested, the treatment was changed according to the randomization assignment. Three randomized replications were completed on Day 1 and two replications were completed on Day 2.

Data was collected from the video recordings by one of the authors (S. Verma), and randomly selected portions were checked by his advisor (V. Lohr). Recorded video was viewed on a computer and time, entries, and falls were recorded. The following time parameters were measured: seconds in each open arm, seconds in each closed arm, and seconds in the center of the maze. A sum of these seconds was used as an additional check on the accuracy of the data collection: data was recollected from the video for any trails that did not sum to exactly 300 seconds (5 minutes). The following entry parameters were also collected: number of entries into each open arm and number of entries into each closed arm. A complete entry was considered

when all four paws were placed inside an arm. The number of times a mouse reached the end of each arm was also recorded. Results for each mouse are presented in the tables in the appendix.

Data analysis

All data were entered into an Excel spreadsheet and then orally and visually crosschecked with the raw data. The percent of entries into an arm was calculated by comparing the number of entries into that arm with the total number of entries into all arms, both open and closed for each mouse. The data for the four mice in one treatment and one replication were averaged to obtain the data for one experimental unit. The averaged data were transferred and analyzed using the Mixed Linear Models Procedure in SAS (SAS Institute Inc., 1999). Linear contrasts were used to compare the means for one treatment with another. A one-tailed test was used to determine significance, because there was a clearly predicted directional response in this study. The standard error (SE) for each mean was calculated by dividing the standard deviation of the mean for the treatment by the square root of the number of mice used for that treatment i.e. n=20.

The very first mouse placed on the maze was mouse-01 from the live plant treatment. It behaved differently from other mouse in the experiment. It entered one closed arm and stayed there for most of time, although it moved from one closed arm to other. When we analyzed mouse-01 data (Figure 5 & 6), it appeared that it never explored open arms. Infect, it stayed inside closed arm-01 for 95% of the treatment time i.e. 5 minutes. However, every other mouse in each treatment explored different arms while in the maze. There might be a possibility that all mice except this first one would have had smell from previous mice present on the maze, since the maze was wiped with a dry towel between mice and not cleaned with alcohol. These might have contributed to the unusual behavior of the first mouse.

Another reason for taking mouse-01 data out could be biological. Because biologically if given a chance to humans, they would at least try each and every corner of the maze once and then depending upon their preferences they would decide which arm to explore more often. But, here in case of mouse-01, it did not behave as a model for the effect of nature on humans. Apart from that when we were analyzing live plant treatment data, mouse-01 readings were not only totally different from other nineteen mice of that treatment, in fact they were different from rest of the 59 mice, based on normal probability plot and box plot results (Using Minitab 15 Student Version) (Figures 5 & 6). It has been found that mouse-01 spent almost all the time into the closed arms. In order to examine the implications of this atypical mouse, the data were analyzed with data from this mouse present (n=60) and with the data from this mouse removed (n=59). Results from both are presented below.

Figure 5 Normality plot and histogram of live plant treatment readings. Mouse-01 readings are different from rest of the readings for closed arm-01(CL1L).

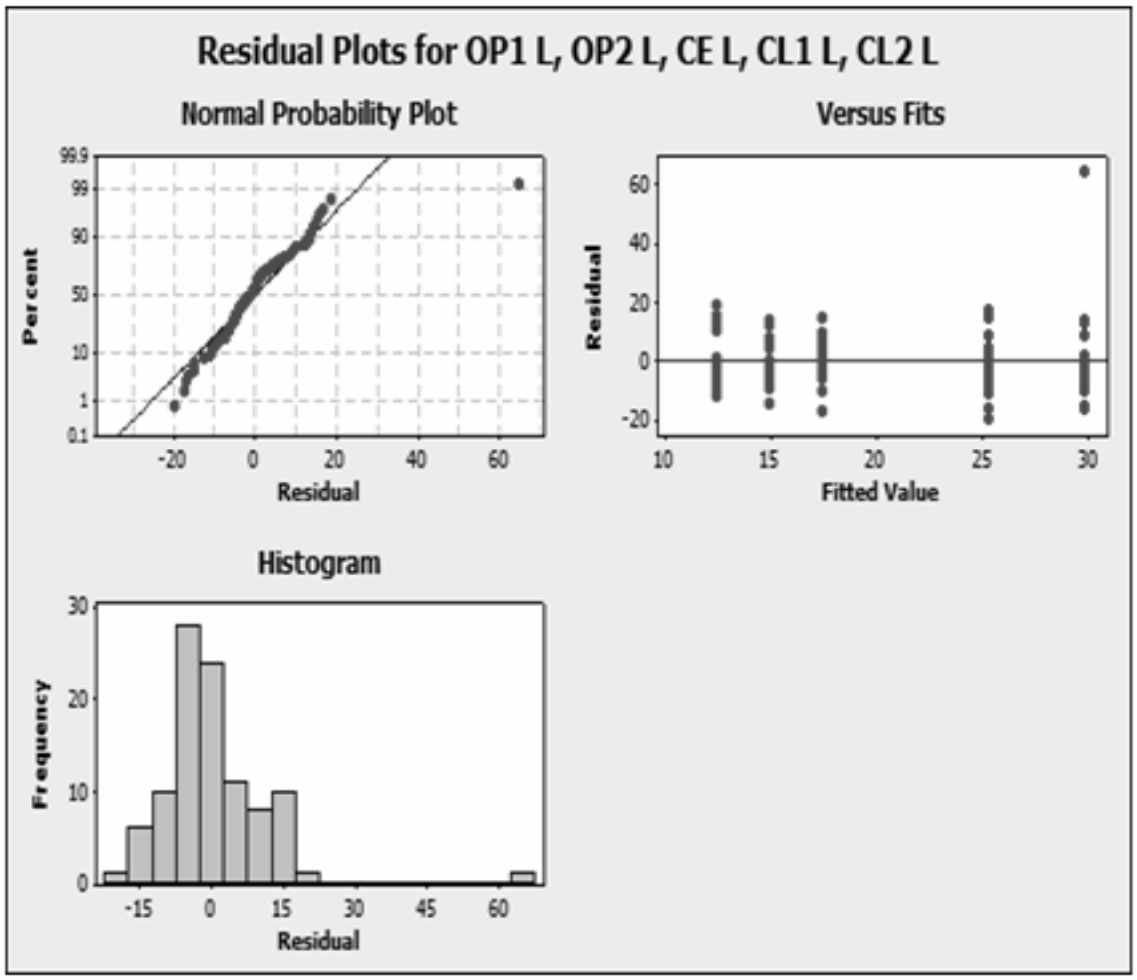
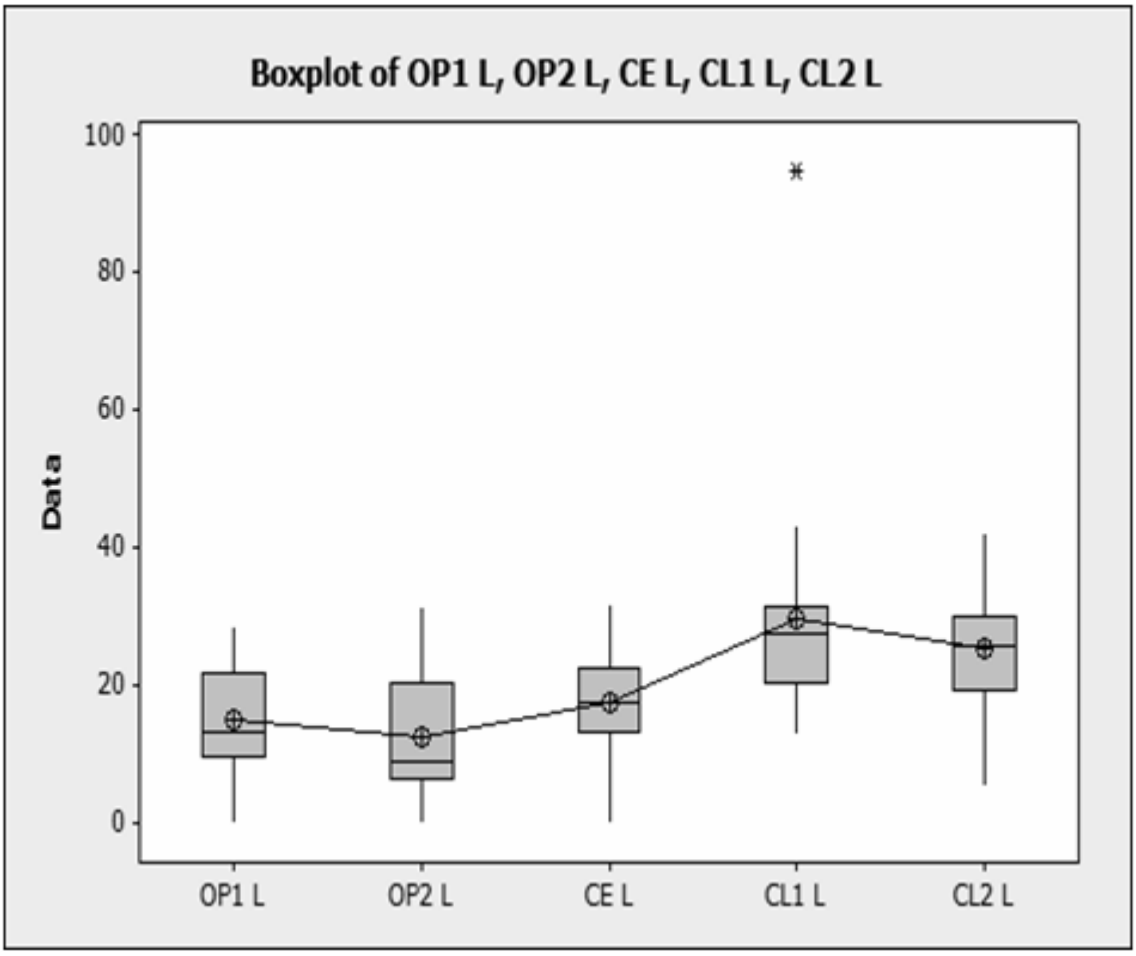


Figure 6 Box plots of live plant treatment readings. Mouse-01 readings are different from rest of the readings for closed arm-01(CL1L).



CHAPTER FOUR

RESULTS

Time spent in the open arms was analyzed with the data for the first mouse removed (Table 2). Mice exposed to live plant environment (n=19) exhibited a statistically significant increase in time spent in the open arms as compared to control mice ($P<0.002$). At the same time, live plant treatment mice (n=19) also spent significantly more time in the open arms compared to artificial plant treatment mice ($P<0.034$). In addition, mice in the artificial plant treatment spent significantly more time in open arms compared to control mice ($P<0.05$). There same comparisons for the time spent in open arms were also all significantly different when analyzed with all 20 mice in the live plant treatment (Table 2.1).

Time spent in the closed arms was also analyzed with the data for the first mouse removed (Table 2). In inverse agreement with the results for time in the open arms, live plant treatment mice (n=19) spent significantly less time in the closed arms as compared to the control mice ($P<0.003$) and artificial plant treatment mice ($P<0.020$). In contrast, time spent in closed arms by artificial plant treatment mice was not statistically different than that of the live plant treatment mice (n=19) ($P<0.146$). Here also, there was no change in what was significantly different for the time spent in closed arms data when analyzed with all 20 mice for live plant treatment (Table 2.1).

Total mean number of entries into open arms made by mice exposed to the live plant environment (n=19) was significantly higher than that of the control mice ($P<0.008$, Table 3), clearly supporting the corresponding time data results (Table 2). The total mean number of entries made by control mice was not statistically different from that of the artificial plant treatment mice ($P<0.069$, Table 3). Similarly, total mean number of entries made by artificial

plant treatment mice was also not statistically different from that of the live plant treatment mice ($P<0.101$). However, when all 20 mice for live plant treatment were included in the analysis, total mean number of entries into the open arms made by artificial plant treatment mice was statistically higher from that of the control mice ($P<0.037$, Table 3.1).

Total mean number of entries into the closed arms made by mice when exposed to live plant environment (n=19) was statistically higher than that of the control mice ($P<0.048$, Table 3). Total number of entries made by control mice was not significantly different from that of the artificial plant treatment mice ($P<0.06$), and similarly there was no significant difference between total mean closed arm entries made by artificial plant treatment mice and live plant treatment mice ($P<0.44$).

The total mean percentage of entries into the open arms made by the live plant treatment mice (n=19) was found to be significantly higher than that of the mice exposed to the control environment (Table 3, $P<0.002$) and also from that of the artificial plant treatment mice ($P<0.017$). However, total mean percentage of entries made by live plant treatment and artificial plant treatment mice was not statistically different ($P<0.106$). When data for all 20 mice the live plant treatment were included in the analysis (Table 3.1), total mean percentage of entries made by artificial plant treatment mice was significantly different from that of the control group ($P<0.037$) whereas results were the same for all other comparisons.

Since there was variability in the ways mice explored the open arms, sometimes going all the way to the end and sometimes less than half way into an open arm, we analyzed the number of times a mouse reached the end of an open arm during each treatment. According to the results, live plant treatment mice data (n=19) was significantly different from control mice

data ($P<0.024$, Table 3). However, artificial plant treatment mice data were not statistically different from control mice data ($P<0.06$) and live plant treatment mice data ($P<0.28$). On the other hand, when data for all 20 mice in the live plant treatment were in the analysis, live plant treatment mice made significantly more full entries into the open arms than control mice ($P<0.018$, Table 3.1). Also, artificial plant treatment mice data were statistically different as compared to control mice data ($P<0.027$).

Further, we noticed the number of mice falling off from the maze during the experiment while taking a U-turn at the end of an open arm during open arm exploration. Eleven out of 20 mice fell down the maze during the control treatment, however only 3 mice fell down during the live plant treatment and 2 mice fell down during the artificial plant treatment. Fernandes and File (1996) suggested that addition of ledges to the open arms could induce more open arm exploration and result in fewer animals falling from maze. They speculated that it could be the openness of the open arms that induced fear/anxiety and also found mice were more anxious without ledges on open arms and less anxious with ledges. In comparison, we also found that during our control treatment mice were most anxious compared to other two treatments. Considering the facts, we here speculate that it might be the anxiogenic property of our control treatment that induced anxiety in mice and hence more falling off.

Data were also separated and analyzed for both n=60 and n=59 on the basis of plant color. Mean entries into open arm, percentage entries into open arm, and mean time spent in open arm (seconds) were analyzed. When analyzed for n=60, all results for green plant were higher than that of the red plant (Table 4), although they were not statistically different. In addition, when n=59 similar trends were true for all the parameters. The interesting finding from this analysis was that although they were not statistically different, but we got a clear direction of

results. All treatment mice were seemed to explore arms having green color plant at the end more often compared to ends having red plant.

Table 2 Total mean time (seconds) spent in the open arms and closed arms by treatment (n=59).

Treatments	Total time in open arms ± SE (Sec)	Total time in closed arms ± SE (Sec)
Control (n=20)	49 ± 2.46	195 ± 3.05
Artificial (n=20)	66 ± 4.39	169 ± 4.28
Live (n=19)	88 ± 3.0	157 ± 5.24
Probability:		
Control vs. Artificial	0.05*	0.020*
Control vs. Live	0.002*	0.003*
Artificial vs. Live	0.034*	0.146^{NS}

*, ^{NS} Probability that the means are significantly different at $P < 0.05$ or not significantly different, respectively.

Table 2.1 Total mean time (seconds) spent in the open arms and closed arms by treatment (n=60).

Treatments	Total time in open arms ± SE(Sec)	Total time in closed arms ± SE (Sec)
Control (n=20)	49±2.46	195±3.05
Artificial (n=20)	67±4.39	169±4.28
Live (n=20)	82±3.00	166±4.70
Probability:		
Control vs. Artificial	0.021*	0.003*
Control vs. Live	0.001*	0.001*
Artificial vs. Live	0.035*	0.304^{NS}

*, ^{NS} Probability that the means are significantly different at $P < 0.05$ or not significantly different, respectively

Table 3 Number and percent of entries made into the open arms and closed arms and number and percent of entries reaching at the end of an open arm by treatment (n=59).

Treatments	Mean entries in open arms ± SE (Number)	Mean entries in closed arms ± SE (Number)	Mean entries in open arms ± SE (% of all open and closed entries)	Mean entries reaching open arm ends ± SE (Number)	Mean entries reaching open arm ends (% of open arm entries)
Control (n=20)	5.20±0.26	18±0.29	21±1.5	2.25±0.12	49
Artificial (n=20)	7.25±0.19	16±0.21	30±0.93	4.10±0.10	52
Live (n=19)	8.98±0.30	16±0.27	35±1.67	4.75±0.31	49
Probability:					
Control vs. Artificial	0.069^{NS}	0.060^{NS}	0.017*	0.060^{NS}	0.367^{NS}
Control vs. Live	0.008*	0.048*	0.002*	0.024*	0.480^{NS}
Artificial vs. Live	0.101^{NS}	0.44^{NS}	0.106^{NS}	0.280^{NS}	0.280^{NS}

*, ^{NS} Probability that the means are significantly different at $P < 0.05$ or not significantly different, respectively

Table 3.1 Number and percent of entries made into the open arms and closed arms and number and percent of entries reaching at the end of an open arm by treatment (n=60).

Treatments	Mean entries in open arms ± SE (Number)	Mean entries in open arms ± SE (% of all open and closed entries)	Mean entries reaching open arm ends ± SE (Number)	Mean entries reaching open arm ends (% of open arm entries)
Control (n=20)	5.20±0.26	21±1.5	2.25±0.12	49
Artificial (n=20)	7.25±0.19	30±0.93	4.10±0.10	52
Live (n=20)	8.35±0.21	33±1.1	4.30±0.21	46
Probability:				
Control vs. Artificial	0.037*	0.007*	0.027*	0.342^{NS}
Control vs. Live	0.006*	0.002*	0.018*	0.320^{NS}
Artificial vs. Live	0.152^{NS}	0.199^{NS}	0.407^{NS}	0.195^{NS}

*, ^{NS} Probability that the means are significantly different at $P < 0.05$ or not significantly different, respectively

Table 4 Data separated based on plant color. Different parameters like mean entries into open arms, % entries into open arms and mean time spent in open arms (seconds) were compared depending upon plant color. Data were also separated based on n=59 and n-60.

	Plant color	Mean entries into open arm	% entries into open arm	Mean time spent in open arm (Seconds)
For n=60	Green Red	4.05 3.75	16.33 15.40	41.60 36.85
Probability		P<0.241	P<0.265	P<0.171
For n=59	Green Red	4.22 3.89	16.95 15.90	42.97 38.33
Probability		P<0.272	P<0.275	P<0.204

CHAPTER FIVE

DISCUSSION

This work has addressed mice behavior and physiology on an elevated plus maze under the influence of environments having live plants, artificial plants, or no plants (control). The maze used in this experiment is very common in the field of neurosciences, psychopharmacology and other anxiety measurement and behavior related studies (Walf et al., 2007). When a mouse was placed on the maze, it was free to explore anywhere without any specific goal to achieve. It has also been noticed that mice seemed to explore randomly from one place to another on the maze. A more anxious mouse was expected to spend more time in the closed arms, where the walls provide some protection. The reason for having three different treatments was to differentiate physiological responses (i.e. anxiety levels) among the surrounding environments of the treatments. Many studies have documented physiological responses to natural environments compared to non-natural environments (Lohr et al., 1996; Ulrich, 1991).

In this experiment we measured total time spent by each mouse in open arms and closed arms. We also measured total percentage of number of entries made by each mouse into open arms and closed arms. We took out first mouse data from live plant treatment (n=19), because it stayed inside the closed arms throughout the 5 minutes of the treatment run and thus had no contribution to the open arms data. No other mouse in the whole experiment had zero contribution to time in the open arms. There may have been previous odors present inside the closed arms that kept this mouse from exploring the open arms. Since we did not used alcohol to clean the floor of the maze, after the first mouse run, other mice might have had a familiar smell

which was not surprising for them and hence they might be more willing to explore the maze. We analyzed the results with and without this mouse (either n=20 or n=19 for live plant treatment).

The results of this study found that mice exposed to the live plant environment spent significantly more time in the open arms compared to control mice (Table 2 & 2.1). They explored the open arms more willingly as compared to control mice, which clearly illustrated that mice felt less stressed in the live plant environment. In addition, live plant treatment mice also spent significantly more time in open arms compared to artificial plant treatment mice. Moreover, artificial plant treatment mice also spent significantly more time in the open arms compared to control mice. There results were in accordance with the findings that a green canopy could have cues that reduce human stress (Kaufman and Lohr, 2008). Data of time spent in the open arms clearly illustrated that all the three treatments were different from each other. The conclusions were similar when the live plant treatment had 19 or 20 mice.

In agreement with time spent in the open arms data, corresponding time spent in the closed arms by live plant treatment mice was statistically lower than that of the control mice. However, time spent in closed arms by live plant treatment mice was not statistically lower than that of the artificial plant treatment mice. At the same time, artificial plant treatment mice spent significantly lower time than that of the control mice inside the closed arms. During both the live plant treatment and the artificial plant treatment, mice went inside the closed arms but did not stay there for a long period of time compared to control mice. Grooming and licking behavior was dominant inside the closed arms during the control treatments. It was noticed that mice often left the closed arms quicker during the live plant treatment and the artificial plant treatment compared to the control treatment. It might be possible that they relaxed within a shorter period

of time due to the presence of plants at the end of closed arms, compared to control group mice with no plants.

Percentage of entries made by each mouse into open arms and closed arms was considered another parameter to analyze anxiety levels in mice during all three treatments. Live plant treatment mice made significantly more percentage of entries into open arms compared to control mice. However, percentage of entries made by live plant treatment mice was not significantly different from that of the artificial plant treatment mice. But, artificial plant treatment mice made significantly more percentage of entries into open arms compared to control mice. There might be something provided by the positioning of plants at the end of each open arms that encouraged the exploration behavior of mice. These results clearly supported corresponding time data results and once again substantiated the valuable findings of other scientists in this area of research that live plants have calming and restorative effects (Kaplan and Kaplan, 1994; Lohr and Pearson-Mims, 2000; Ulrich, 1981, 1984). These results potentially were due to the obvious explanation that mice were more willing to go and explore the open arms not only during the live plant treatment but also during the artificial plant treatment compared to the control treatment. The conclusion was similar when the live plant treatment had 19 or 20 mice.

In order to estimate intensity of willingness of mice to explore the open arms during each treatment, we analyzed how many times a mouse went all the way to the end of an open arm during each treatment. Interestingly, live plant treatment mice made more entries all the way to the end of open arms compared to control mice ($p<0.024$), but full entries made by artificial plant treatment mice was not statistically different from control mice and live plant treatment mice.

Although, these results were in the direction of our proposed hypothesis, surely we need a better animal model in order to distinguish each treatment significantly on this parameter.

According to the results of this experiment, mice were less stressed and willing to explore the open arms more often when exposed to the live plants environment. These results reinforced previous findings that simply viewing green natural settings through windows after surgery in a hospital is able to produce faster recovery in humans (Ulrich, 1984). In another parallel study, Lohr at al. (2000) tested physical discomfort levels in different working environments having live green plants, no plants and non-plant objects. Subjects were asked to keep their hands in ice water for 5 minutes. At the end of the experiment it was found that more subjects with live plants were able to keep their hands in ice cool water for the full period of time than other groups. In our experiment, although we found consistent significant results for all three treatments when total time spent and total entries made into open arms was analyzed, the difference between artificial plant treatment mice and live plant treatment mice results were not significant enough to make a clear statement that live plant environment was more stress reducing than artificial plant environment.

Mice of control group exposed themselves minimally to the open arms. It might be possible that they didn't find anything within their visibility when trying to explore the open arms that could actually hold their attention and minimize risks and cues associated with the environment. In contrast, the author noticed that when mice from live plant treatment and artificial plant treatment tried to come out of the closed arms, they appeared to find something within their visibility that encouraged them to explore open arms. This finding was in accordance with the theory proposed by Kaplan and Kaplan (1989), which explains the idea of complexity and mystery. Thus, it can be said on the basis of these results that plants, whether live or

artificial, were able to attract mice towards them and encouraged exploration behavior. It might be possible that it was the color of the plants that contributed to these findings.

When it appeared that mice may have preferred movement to the open arms having a green plant at the end, we separated the data on the basis of plant's color. Interestingly, we found that mice made substantially more entries into open arms that had green colored plants compared to open arms having red colored plants at the end of open arm. After analyzing data on the basis of the plant's color, we came to the conclusion that there might be a possible role of plant color in the whole findings. These results were in parallel with studies suggesting that people were more relaxed when viewing green tree canopies compared to red, yellow or orange colored canopies (Kaufman and Lohr, 2008). It appears from these results that plant color could be a determining factor in making decisions for certain types of preferences: green color seems to work better than other colors.

It was common that control treatment was significantly different from other treatments and sometimes artificial plant treatment was significantly different from live plant treatment. The possible speculated reason for the difference between live plant treatment and artificial plant treatment results could be the ability of live green plants to reflect green color. Leaves of live plants can reflect green color of leaves better because of the presence of cuticle layer, whereas dry leaves of artificial plants might not be able to reflect foliage color well. Consequently, mice of live plant treatment could see more green foliage color during live plant treatment compared to artificial plant treatment. In addition, this phenomenon might lead them to make an imaginary frame of reference at each end of the open arms and, in turn result in more exploration behavior into open arms. Apart from that mice of live plant treatment were found to be more relaxed compared to artificial plant treatment mice and control as well. It could be possible, since live

plants can do photosynthesis, and hence more fresh air around the maze could be one of the reasons for getting more relaxed behavior during live plant treatment.

Finally, results of this experiment substantiated the notion that people feel less anxious when their surrounding environment contains live green plants compared to inanimate objects or no objects. Further, based on the results of this study we can say that mice response to the plants was innate and very likely human response to plants might be innate.

CHAPTER SIX

CONCLUSION

There is ample evidence that humans response innately to nature. This study was designed in order to see if naïve mice also respond innately to nature. In this study, mice responded statistically differently towards settings having live plants or artificial plants compared to no plants (control). In this elevated plus-maze study, naïve mice were found to be less stressed when exposed to a live plant environment or an artificial plant environment as compared to a no plant environment. These results were in agreement with the findings of Ulrich (1979, 1984) and Lohr and others (1996).

Although we did not measure the direct role of live or artificial plant color in this anxiety alleviating phenomenon, there seemed to be a possible role of the plant's color in this experiment. Kaufman and Lohr (2008) also documented a significant effect of plant's color on human physiology. In this study mice appeared to be more attracted towards green colored plants compared to red colored plants. These results paralleled the findings of Kaufman and Lohr (2008), where people were calmer when looking at trees with green canopies. However, there were no significant differences in response to color between live plant treatment mice and artificial plant treatment mice. It might be the color or the coverage provided by the plants that generated possible shelter like appearance in the illuminated environment that in turn, appealed to the animals and encouraged them to explore the maze. Since rodents are nocturnal animals and always explore things in darkness (Clive Roots, 2006), which is also evident from the fact that mice spent maximum time (seconds) in closed arms during control group treatment where no other color cues were available except darkness provided by the closed arms, this supposition is

reasonable. However, as we go from the artificial plant treatment (169 seconds) to the live plant treatment (157 seconds) time spent in the closed arms decreased significantly compared to control group (195 seconds) and on the other hand, open arms exploration time increased significantly. These results suggest that there might be something provided by the positioning of plants at the end of each open arms that encouraged the exploration behavior of mice.

The mouse has a more developed olfactory sense than the human has (Quignon et al., 2003). There might be some contribution of odor in this experiment. On the contrary, during our preliminary experiment with rats, we used ornamental millet having a strong odor as one of the plants, and there was no evidence that the rats were more attracted towards it.

Although this animal model was not able to differentiate significantly between the live plant treatment and the artificial plant treatment, except time spent in open arms ($P<0.034$), it can be concluded that naïve mice were less stressed and attracted more towards the live plant environment and this phenomena of attraction was innate. This experiment once again substantiated the notion that live plant environment is able to alleviate stress levels in humans. At the same time more research in this area is needed.

For more reliability and in order to quantify the evidence associated with each and every physiological change, one has to have some sort of equipment that can precisely measure either brain activity or hormonal activity during real time in an experiment. Obtaining direct physiological evidence could be possible if one could assay corticotrophin releasing hormone non-invasively (Gunnar and Quevedo, 2007). Direct physiological evidence could also be possible if somebody could quantify stress hormones (corticosterone) during the experiment.

In order to find out the effect of light and have a more controlled experiment, one could do the whole experiment in different light environment i.e. green light, blue light or UV light. That way we can clearly distinguish mice behavior from one treatment to that of the other. Apart from that biochemical estimation of stress hormones could also be helpful. In addition, now at WSU in the Department of Electrical Engineering and Computer Science, electronic chips are available which can actually estimate brain activity of rodents during their exploration and can estimate brain activity of rodents during the experiment. An animal model that could control all variables, such as smell, light, and color that could affect the results of an experiment, might be the one we would like to look forward to. Finally, we here propose that rodents might have innate perception towards green color of live plant and artificial plant setting which is able to alleviate anxiety.

REFERENCES

Antoniou, K. G., Papathanasiou, G. Panagis, G. Nomikos, T. Hyphantis & Z. Papadopoulou-Daifoti (2004). Individual responses to novelty predict qualitative Differences in d - amphetamine-induced open field but not Reward-related behaviors in rats. *Neuroscience*, 1230, 613-623.

Appleton, J. (1975a). *The experience of landscape.* New York, NY: John Wiley**.**

Appleton, J. (1975). Landscape evaluation: The theoretical vacuum. *Transactions of the Institute of British Geographers,* 66, 120-123.

Balling, J. D., & Falk, J. H. (1982). Development of visual preferences for natural environments. Environment and Behavior, 14, 5-28

Berlyne, D. E. (1971). *Aesthetics and psychology.* New York: Appleton-Century-Crofts

Butler, P. D., Weiss, J. M., Stout J. C., & Nemeroff, C. B. (1990). Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J. Neurosci.,* 10, 176-183.

Cimprich, B. (1993). Development of an intervention to restore attention on cancer patients. *Cancer Nursing,* 16(2), 83-92.

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of stress response. *Annu. Rev. Physiol*., 67, 259-284.

Dawson, G. R. & Tricklebank, M. D. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents. *Current Techniques*, *TiPS*, 16, 33-36.

de Kloet, E. R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Front. Neuroendocrinol*., 14, 495-518.

de Kloet, E. R., Rots, N. Y., & Cools, A. R. (1996). Brain-corticosteroids hormone dialogue: Slow and persistent. *Cell. Mol. Neurobiol.,* 16, 345-56.

Dijkstra, K., Pieterse, M., & Pruyn, A. (2006). Physical environmental stimuli that turn healthcare facilities into healing environments through psychologically mediated effects: Systematic review. *J. Adv. Nurs.*, 56, 166–181.

Dijkstra, K., Pieterse, M. E., & Pruyan, A. (2008). Stress reducing effects on indoor plants in the built healthcare environment: The mediating role of perceived attractiveness. *Preventive Medicine*, 47, 279-283.

Diette, G. B., Haponik, E., Devrotes, A., & Rubin, H. R. (2003). Distraction therapy with nature sights and sounds reduces pain during flexible bronchoscopy: A complete approach to routine analgesia. *Chest*, 123(3), 941-948.

Doyle, S., & Menaker, M. (2007). Circadian photoreception in vertebrates. *Cold Spring Harb. Symp. Quant Biol.,* 72, 499-508.

Elfline, G. S., Branda, E. M., Babich, M. & Quock, R. M. (2004). Antagonism by NOS Inhibition of the Behavioral Effects of Benzodiazepine and $GABA_A$ Receptor Agonists in the Mouse Elevated Plus-Maze. *Neuropsychopharmacology*, 29, 1419-1425

Fernandes, C. & File, S. E (1996). The Influence of Open Arm Ledges and Maze Experience in the Elevated Plus-Maze. *Pharmacology Biochemistry and Behavior*, 54(1), 31-40.

Flagler, J. (1995). Role of Horticulture in training correctional youth. *Hort Technology,* 5, 185-187.

Gunnar, M., & Quevedo, K. (2007). The neurobiology of stress and development. *Annu. Rev. Psychol.*, 58, 145-73.

Hecht, E. (1987). *Optics*. 2nd Ed, Addison Wesley.

Heerwagen, J. H. (1990). Psychological aspects of windows and window design, In R. I. Selby, K. H. Anthony, J. Choi, & B. Orland, Eds., *Preceedings of the 21st Annual Conference of the Environmental Design & Research Association* (pp.269-280). Oklahoma City: EDRA

Heinrichs, S. C., Menzaghi, F., Pich, E. M., Britton, K. T., & Koob, G. F. (1995). The role of CRF in behavioral aspects of stress. *Ann. NY Acad. Sci.*, 771, 92-104.

Hinojosa, F. R., Spricigo Jr. L., Izidio, G. S., Bruske, G. R., Lopes, D. M. & Ramos, A. (2006). Evaluation of two genetic animal models in behavioral tests of anxiety and depression. *Behavioural Brain Research*, 168, 127–136.

Izidio, G. S., Spricigo, Jr., L. & Ramos, A. (2005). Genetic differences in the elevated plus-maze persist after first exposure of inbred rats to the test apparatus. *Behavioural Processes*, 68, 129–134.

Jacobs, G. H., Fenwick, J. A., & Williams, G. A. (2001). Cone-based vision of rats for ultraviolet and visible lights. *Journal of Experimental Biology*, 204(14), 2439-2446.

Jacobs, G.H., Neitz, J., & Deegan, J.F. II., (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature*, 353, 655-656.

Joye, Y. (2007). Architectural lessons from environmental psychology: The case of biophilic architecture. *Review of General Psychology*, 11(4), 305-328.

Kalynchuk, L. E., Pinel, J. P. J., Treit, D. & Kippin, T. E. (1997). Changes in emotional behavior produced by long term amygdala kindling in rats. *Biological Psychiatry*, 14, 438-451.

Kaplan, R. (1973). Some psychological benefits of gardening. *Environ. Behavior.*, 5, 145-162.

Kaplan, R., & Kaplan, S. (1989). *The experience of nature: A psychological perspective.* Cambridge, England: Cambridge University Press.

Kaplan, S. (1987). Aesthetics, affect and cognition. *Environment and Behavior, 19,* 3–32.

Kaplan, S. (1988). Perception and landscape: Conceptions and misconceptions. In J. Nasar, (Ed.), *Environmental aesthetics: Theory, research, and applications* (pp. 45–55). Cambridge, England: Cambridge University Press.

Kaufman, A. J. & Lohr, V. I. (2008). Does it Matter What Color Tree You Plant? In *Proc. VIIIth Int. People-Plant Symp.* Eds, Matsua, E., Relf, P. D. & Burchett, M, Acta Hort., 790, 179-184.

LaVail, M. M. (1976). Survival of some photoreceptors in albino rats following long-term exposure to continuous light. *Invest. Ophthalmol. Vis. Sci.*, 15, 64-70.

Lohr, V. I., Pearson-Mims, C. H., & Goodwin, G. K. (1996). Interior plants improve worker productivity and reduces stress in a windowless environment. *J. Environ. Hort.*, 14(2), 97-100.

Lohr, V. I. & Pearson-Mims, C. H. (2000). Physical discomfort may be reduced in the presence of interior plants. *HortTechnology*, 10(1), 53-58.

Lohr, V. I., & Relf, P. D. (2000). An overview of the current state of human issues in horticulture in the United States. *HortTechnology*, 10(1), 27-33.

Lohr, V. I., & Pearson-Mims, C. H. (2002). Childhood contact with nature influences adult attitudes and actions towards trees and gardening. In *Interaction by Design*, C. A. Shoemaker (Ed.) Iowa State Press; Ames, Lowa, pp-267-277.

Lohr, V. I., & Pearson-Mims, C. H. (2006). Responses to scenes with spreading, rounded, and conical tree forms. *Environment and Behavior*, 38(5), 667-688.

Montegmory, K. C. (1955). *Journal of Comparative Physiology and Psychology*. 48, 254-260

Moore, E. O. (1982). A prison environment's effect on health care service demands. *Journal of Environmental Systems*, 11, 17-34.

Nelson, D. A., & Marler, P. (1990). The perception of birdsong and an ecological concept of signal space. In Stebbins, W.C. & Berkley, M.A. eds, *Comparative Perception*, 2: Complex Signals. pp, 443 - 478. New York: John Wiley.

Newman, L.A., Walker, M. T., Brown, R. L., Cronin, T. W., & Robinson, P. R. (2003). Melanopsin forms a functional short-wavelength photopigment. *Biochemistry*, 42 (44), 12734-12738. Retrieved from http://pubs.acs.org on February 26, 2009.

Olmsted, F. L. (1865). The value and care of park. Report to the Congress of the state of California. *The American Environment*. Reading, MA : Addison-Wesley, 18-24.

Parson, R. (1991). The potential influences of environmental perception on human health. *Journal of Environmental Psychology*, 11, 1-23

Palkovits, M. (1987). Organization of the stress response at the anatomical level. *Brain Research*, ed. de Kloet, E. R., Wiegant, V.M., de Wied, D., *Amsterdam: Elsevier Sci.*, 47-55.

Quignon, P., Kirkness, E., Cadieu, E., Touleimat, N., Guyon, R., Renier, C., Hitte, C., André, C., Fraser, C., & Francis Galibert (2003). Comparison of the canine and human olfactory receptor gene repertoires. *Genome Biology*, 4. R:80

Radlwimmer, F. B., & Yokoyama, S. (1998). Genetic analyses of the green visual pigments of rabbit (Oryctolagus cuniculus) and rat (Rattus norvegicus). *Gene*. 218(1-2), 103-9.

Rodgers, R. J. & Dalvi, A. (1997). Anxiety, Defence and the Elevated Plus-maze. Neuroscience and Behavioral Reviews, 21(6), 801-810

Roots, C. (2006). Nocturnal Chisellers. In *Nocturnal Animals*. Greenwood Publishing Group, 139-142

Russell, S. (December 05, 2002). Of mice and men/ Striking similarities at the DNA level could aid research. Retrieved March 17, 2009, from http://www.sfgate.com/cgi-bin/article.cgi?f=/c/a/2002/12/05/MN153329.DTL&type=science.

SAS Institute Inc., (1999). The *Mixed* procedure. In SAS/STAT User's guide, Version 8, Vol. 2. Cary, NC: SAS Institute.

Sanchez, M. M., Young, L. J., Plotsky, P. M., & Insel, T. R. (2000). Distribution of corticosteroid receptors in the rhesus brain: Relative absence of glucocorticoid receptors in the hippocampal formation. *J. Neurosci.*, 20, 4657-4768.

Sapolsky, R. M. (1997). McEwen-induced modulation of endocrine history: A partial review. *Stress,* 2, 2-12.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21:55–89.

Spencer, G., (September, 2007). Background on Mouse as a Model Organism. Retrieved March 16th, 2009, from www.genome.gov/10005834.

Stanbrook, E. (1968). Human needs and the natural environments. In *Man and Nature in the City*. Proceedings of Symposium sponsored by the Bureau of Sports Fisheries and Wildlife. Washington, DC: US Department of the Interior, 1-9.

Taylor, A. F., Kuo, F. E. &, Sullivan, W. C. (2001). Coping with ADD: The surprising connection to green play settings. *Environment and Behavior*, 33(1), 54-77.

Tennessen, C. M. & Cimprich, B. (1995). Views to nature: Effects on attention. *Journal of Environmental Psychology*, 15, 77-85.

Ulrich, R. S. (1979). Visual landscapes and psychological well-being. *Landscape Res*., 4, 17-23.

Ulrich, R. S. (1981). Natural versus urban scenes-some psychophysiological effects. *Environment and Behavior, 13,* 523–556.

Ulrich, R. S. (1983). Aesthetic and affective response to natural environment. In I. Altman & J. F. Wohlwill (Eds.), *Human behavior and the environment,* 6, 85–125. New York: Plenum Press.

Ulrich, R. S. (1984). View through a window may influence recovery from surgery. *Science, 224,* 420–421.

Ulrich, R. S. (1986). Human Responses to Vegetation and Landscapes. *Landscape and Urban Planning*, 13, 29-44

Ulrich, R. S. (1986). Effects of hospital environments on patient well-being (Research rep. 9[55]). Trondheim, Norway: Department of Psychiatry and Behavioral Medicine, University of Trondheim.

Ulrich, R.S. & Parsons, R. (1990). Influences of passive experiences with plants on individual well-being and health. Paper presented at the *National Symposium on the Role of Horticulture in Human Well- Being and Social development*, Washington, D.C.

Ulrich, R. S., Simons, R. F., Losito, B. D., Fiorito, E., Miles, M. A., & Zelson, M. (1991). Stress recovery during exposure to natural and urban environments. *Journal of Environmental Psychology, 11,*201–230.

Ulrich, R. S. (1993). Biophilia, biophobia, and natural landscapes. In S. R. Kellert & E. O. Wilson (Eds.), *The biophilia hypothesis*, 73–137. Washington, DC: Island Press.

Ulrich, R. S., & Zimring, C. (2004). The role of the physical environment in the hospital of the 21st century: A once-in-a-lifetime opportunity. Retrieved March 11, 2009, from http://www.healthdesign.org/research/reports/pdfs/role_physical_env.pdf.

University of Virginia (2008, August 15). Light receptors in eyes play key role in setting biological clock. Health and Medicine, Retrieved March, 23, 2009 from http://esciencenews.com/articles/2008/08/15/light.receptors.eye.play.key.role.setting.biological.clock.study.shows

Verderber, S (1986). Dimensions of person-window transactions in the hospital environment. *Environment and Behavior*, 18, 450-466.

Wager-Smith, K. & Kay, S.A. (2000). Circadian rhythm genetics: From flies to mice to humans. *Nature genetics*, 26, 23-27.

Walf, A. A. & Frye, C. A. (2007). The use of elevated plus-maze as an essay of anxiety-related behavior in rodents. Nature. doi:10.1038/nprot.44.

Wall, P. M. & Messier, C. (2001). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neuroscience and Behavioral Reviews, 25, 275-286

Wohlwill, J. F. (1976). Environmental aesthetics: The environment as a source of affect. In I. Altman & J. F. Wohlwill, Eds., *Human Behavior and Environment*, New York: Plenum, 1, 37-86.

Wohlwill, J. F. (1983). The concept of nature: A psychologist's view. In I. Altman & J. F. Wohlwill, Eds., *Human Behavior and Environment*, New York: Plenum Press, 6, *Behavior and the Natural Environment*, 5-37.

APPENDIX

Table A1 Time (seconds) spent by each mouse in the control group in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Control group (seconds)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	18	18	58	135	69
2	41	41	65	90	61
3	16	16	45	70	131
4	16	16	24	106	143
5	21	21	24	124	124
6	3	3	23	176	88
7	16	16	133	103	0
8	30	30	70	105	90
9	28	28	72	71	78
10	12	12	20	95	155
11	58	58	69	40	90
12	36	36	84	75	88
13	31	31	33	107	119
14	24	24	56	79	98
15	19	19	37	100	127
16	35	35	55	91	109
17	47	47	65	101	52
18	18	18	76	114	82
19	30	30	53	106	100
20	20	20	50	101	112

Table A2 Percentage time spent by each mouse in the control group in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Control Group (% time)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	6	6.6	19.3	45	23
2	13.6	14.3	21.6	30	20.3
3	5.3	12.6	15	23.3	43.6
4	5.3	3.6	8	35.3	47.6
5	7	2.3	8	41.3	41.3
6	1	3.3	7.6	58.6	29.3
7	5.3	16	44.3	34.3	0
8	10	1.6	23.3	35	30
9	9.3	17	24	23.6	26
10	4	6	6.6	31.6	51.6
11	19.3	14.3	23	13.3	30
12	12	5.6	28	25	29.3
13	10.3	3.3	11	35.6	39.6
14	8	14.3	18.6	26.3	32.6
15	6.3	5.6	12.3	33.3	42.3
16	11.6	3.3	18.3	30.3	36.3
17	15.6	11.6	21.6	33.6	17.3
18	6	3.3	25.3	38	27.3
19	10	3.6	17.6	35.3	33.3
20	6.6	5.6	16.6	33.6	37.3

Table A3 Total percentage time spent by each mouse in the control group in the closed arms, the open arms and the center of maze

Control group (Total % time)			
Mice No.	Open arms	Center	Closed arms
1	12.67	19.34	68
2	28	21.67	50.34
3	18	15	67
4	9	8	83
5	9.34	8	82.67
6	4.34	7.67	88
7	21.34	44.34	34.34
8	11.67	23.34	65
9	26.34	24	49.67
10	10	6.67	83.34
11	33.67	23	43.34
12	17.67	28	54.34
13	13.67	11	75.34
14	22.34	18.67	59
15	12	12.34	75.67
16	15	18.34	66.67
17	27.34	21.67	51
18	9.34	25.34	65.34
19	13.67	17.6667	68.67
20	12.34	16.67	71

Table A4 Time (seconds) spent by each mouse in the artificial plant treatment in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Artificial plant (Seconds)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	44	39	55	99	63
2	8	22	82	86	102
3	44	24	33	115	84
4	4	0	51	125	120
5	12	18	93	71	106
6	34	11	73	98	84
7	24	6	82	74	114
8	38	44	69	51	98
9	36	32	58	72	102
10	40	15	75	69	101
11	113	28	61	53	45
12	49	25	64	76	86
13	36	6	66	84	108
14	38	32	54	123	53
15	52	39	58	77	74
16	51	26	62	66	95
17	59	62	47	47	85
18	38	29	58	61	114
19	8	25	62	91	114
20	68	58	73	73	28

Table A5 Percentage time spent by each mouse (n=19) of artificial plant treatment in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Artificial plant (% time)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	14.67	13	18.34	33	21
2	2.67	7.34	27.34	28.67	34
3	14.67	8	11	38.34	28
4	1.34	0	17	41.67	40
5	4	6	31	23.67	35.34
6	11.34	3.67	24.33334	32.67	28
7	8	2	27.34	24.67	38
8	12.67	14.67	23	17	32.67
9	12	10.67	19.34	24	34
10	13.34	5	25	23	33.67
11	37.67	9.34	20.34	17.67	15
12	16.34	8.34	21.34	25.34	28.67
13	12	2	22	28	36
14	12.67	10.67	18	41	17.67
15	17.34	13	19.34	25.67	24.67
16	17	8.67	20.67	22	31.67
17	19.67	20.67	15.67	15.67	28.34
18	12.67	9.67	19.34	20.34	38
19	2.67	8.34	20.67	30.34	38
20	22.67	19.34	24.34	24.34	9.34

Table A6 Total percentage time spent by each mouse in the artificial plant treatment in the closed arms, the open arms and the center of maze

Artificial plant (Total % time)			
Mice No.	Open arms	Center	Closed arms
1	27.67	18.34	54
2	10	27.34	62.67
3	22.67	11	66.34
4	1.34	17	81.67
5	10	31	59
6	15	24.34	60.67
7	10	27.34	62.67
8	27.34	23	49.67
9	22.67	19.34	58
10	18.34	25	56.67
11	47	20.34	32.67
12	24.67	21.34	54
13	14	22	64
14	23.34	18	58.67
15	30.34	19.34	50.34
16	25.67	20.67	53.67
17	40.34	15.67	44
18	22.34	19.34	58.34
19	11	20.67	68.34
20	42	24.34	33.67

Table A7 Time (seconds) spent by each mouse in the live plant treatment in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Live plant (Seconds)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	0	0	0	284	16
2	67	94	53	44	42
3	29	35	41	127	68
4	82	36	56	59	67
5	37	24	33	83	123
6	62	28	39	44	127
7	31	26	34	130	79
8	35	16	57	89	103
9	23	23	65	69	120
10	58	18	95	69	60
11	28	68	71	84	49
12	44	38	60	82	76
13	30	78	20	86	86
14	16	5	79	115	85
15	36	11	68	95	90
16	86	77	40	39	58
17	68	39	53	66	74
18	42	22	81	74	81
19	81	84	48	60	27
20	43	24	53	91	89

Table A8 Percentage time spent by each mouse in the live plant treatment in open arm-01, open arm-02, closed arm-01, closed arm-02 and center of the maze.

Live plant (% time)					
Mice no.	Open arm-01	Open arm-02	Center	Closed arm-01	Closed arm-02
1	0	0	0	94.67	5.34
2	22.34	31.34	17.67	14.67	14
3	9.67	11.67	13.67	42.34	22.67
4	27.34	12	18.67	19.67	22.34
5	12.34	8	11	27.67	41
6	20.67	9.34	13	14.67	42.34
7	10.34	8.67	11.34	43.34	26.34
8	11.67	5.34	19	29.67	34.34
9	7.67	7.67	21.67	23	40
10	19.34	6	31.67	23	20
11	9.34	22.67	23.67	28	16.34
12	14.67	12.67	20	27.34	25.34
13	10	26	6.67	28.67	28.67
14	5.34	1.67	26.34	38.34	28.34
15	12	3.67	22.67	31.67	30
16	28.67	25.67	13.34	13	19.34
17	22.67	13	17.67	2	24.67
18	14	7.34	27	24.67	27
19	27	28	16	20	9
20	14.34	8	17.67	30.34	29.67

Table A9 Total percentage time spent by each mouse in the live plant treatment in the closed arms, the open arms and the center of maze

Live plant (Total % time)			
Mice No.	Open arm	Center	Closed arm
1	0	0	100
2	53.67	17.67	28.67
3	21.34	13.67	65
4	39.34	18.67	42
5	20.34	11	68.67
6	30	13	57
7	19	11.34	69.67
8	17	19	64
9	15.34	21.67	63
10	25.34	31.67	43
11	32	23.67	44.34
12	27.34	20	52.67
13	36	6.67	57.34
14	7	26.34	66.67
15	15.67	22.67	61.67
16	54.34	13.34	32.34
17	35.67	17.67	46.67
18	21.34	27	51.67
19	55	16	29
20	22.34	17.67	60

Table A10 Number of entries made by each mouse in the control group into open arm-01, open arm-02, closed arm-01, closed arm-02 and all arms (total)

Control Group (Number of Entries)					
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02	Total entries
1	1	5	14	8	28
2	3	5	11	8	27
3	3	2	6	8	19
4	3	1	8	11	23
5	1	1	7	5	14
6	0	2	13	10	25
7	0	1	9	8	18
8	3	1	5	6	15
9	6	4	8	11	29
10	1	2	8	9	20
11	7	4	4	8	23
12	3	4	12	10	29
13	1	1	10	12	24
14	2	3	8	10	23
15	1	2	14	17	34
16	4	1	8	9	22
17	9	6	10	7	32
18	3	1	13	11	28
19	3	1	9	7	20
20	2	1	7	9	19

Table A11 Percentage of entries made by each mouse in the control group in open arm-01, open arm-02, closed arm-01, and closed arm-02 of the maze.

Control group (% Entry)				
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02
1	3.57	17.85	50	28.57
2	11.11	18.51	40.74	29.62
3	15.78	10.52	31.57	42.10
4	13.04	4.34	34.78	47.82
5	7.14	7.14	50	35.71
6	0	8	52	40
7	0	5.55	50	44.44
8	20	6.66	33.34	40
9	20.68	13.79	27.58	37.93
10	5	10	40	45
11	30.43	17.39	17.39	34.78
12	10.34	13.79	41.37	34.48
13	4.16	4.16	41.67	50
14	8.69	13.04	34.78	43.47
15	2.94	5.88	41.17	50
16	18.18	4.54	36.36	40.90
17	28.12	18.75	31.25	21.87
18	10.71	3.57	46.42	39.28
19	15	5	45	35
20	10.52	5.26	36.83	47.36

Table A12 Number of entries made by each mouse in the artificial plant treatment group into open arm-01, open arm-02, closed arm-01, closed arm-02 and all arms (total)

Artificial plant treatment (Number of entries)					
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02	Total entries
1	4	6	10	7	27
2	3	3	8	9	23
3	4	4	7	6	21
4	1	0	9	12	22
5	5	5	6	10	26
6	5	6	9	10	30
7	4	1	6	9	20
8	4	4	7	9	24
9	3	2	9	10	24
10	5	2	8	12	27
11	8	3	8	5	24
12	4	2	9	10	25
13	3	2	9	11	25
14	2	3	7	4	16
15	4	4	11	8	27
16	3	3	7	8	21
17	4	6	4	7	21
18	4	3	7	8	22
19	1	2	6	12	21
20	6	7	8	5	26

Table A13 Percentage entry made by each mouse in the artificial plant treatment group in open arm-01, open arm-02, closed arm-01, and closed arm-02 of the maze.

Artificial plant treatment (% Entry)				
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02
1	14.81	22.22	37.03	25.92
2	13.04	13.04	34.78	39.13
3	19.04	19.04	33.34	28.57
4	4.54	0	40.90	54.54
5	19.23	19.23	23.07	38.46
6	16.67	20	30	33.34
7	20	5	30	45
8	16.67	16.67	29.16	37.5
9	12.5	8.34	37.5	41.67
10	18.51	7.40	29.62	44.44
11	33.34	12.5	33.34	20.83
12	16	8	36	40
13	12	8	36	44
14	12.5	18.75	43.75	25
15	14.81	14.81	40.74	29.62
16	14.28	14.28	33.34	38.09
17	19.04	28.57	19.04	33.34
18	18.18	13.63	31.81	36.36
19	4.76	9.52	28.57	57.14
20	23.07	26.92	30.76	19.23

Table A14 Number of entries made by each mouse in the live plant treatment group into open arm-01, open arm-02, closed arm-01, closed arm-02 and all arms (total)

Live plant treatment (Number of entries)					
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02	Total entries
1	0	0	3	2	5
2	9	9	7	7	32
3	2	3	6	11	22
4	6	9	7	5	27
5	4	1	6	9	20
6	5	2	4	13	24
7	3	4	13	10	30
8	2	4	7	9	22
9	3	2	8	5	18
10	5	6	5	6	22
11	4	3	11	5	23
12	4	3	8	9	24
13	5	4	11	10	30
14	3	1	14	10	28
15	4	2	10	9	25
16	6	6	3	6	21
17	8	9	9	8	34
18	3	3	8	11	25
19	7	8	5	3	23
20	2	3	11	10	26

Table A15 Percentage entry made by each mouse in the live plant treatment group in open arm-01, open arm-02, closed arm-01, and closed arm-02 of the maze.

Live Plant Treatment (% entry)				
Mice no.	Open arm-01	Open arm-02	Closed arm-01	Closed arm-02
1	0	0	60	40
2	28.12	28.12	21.87	21.87
3	9.09	13.63	27.27	50
4	22.22	33.34	25.92	18.51
5	20	5	30	45
6	20.83	8.34	16.67	54.16
7	10	13.34	43.34	33.34
8	9.09	18.18	31.81	40.90
9	16.67	11.11	44.44	27.77
10	22.72	27.27	22.72	27.27
11	17.39	13.04	47.82	21.73
12	16.67	12.5	33.34	37.5
13	16.67	13.34	36.67	33.34
14	10.71	3.57	50	35.71
15	16	8	40	36
16	28.57	28.57	14.28	28.57
17	23.52	26.47	26.47	23.52
18	12	12	32	44
19	30.43	34.78	21.73	13.04
20	7.69	11.53	42.30	38.46

Table A16 Total number of entries made by each mouse in the control group into open arm-01 and open arm-02 and number and percentage of times a mouse reached the end open arm-01 and open arm-02

Control Group						
	Number of entries	Number of entries	Entries reached to the end	Entries reached to the end	% of entries reached to the end	% of entries reached to the end
Mice No.	Open arm-01	Open arm-02	Open arm-01	Open arm-02	Open arm-01	Open arm-02
1	1	5	1	1	16.67	16.67
2	3	5	3	2	37.5	25
3	3	2	2	2	40	40
4	3	1	1	1	25	25
5	1	1	1	1	50	50
6	0	2	0	1	0	50
7	0	1	0	1	0	100
8	3	1	1	0	25	0
9	6	4	3	0	30	0
10	1	2	1	0	33.34	0
11	7	4	4	3	36.34	27.23
12	3	4	1	2	14.21	28.53
13	1	1	1	0	50	0
14	2	3	1	1	20	20
15	1	2	1	1	33.34	33.34
16	4	1	0	0	0	0
17	9	6	1	2	6.67	13.34
18	3	1	0	1	0	25
19	3	1	1	1	25	25
20	2	1	1	1	33.34	33.34

Table A17 Total number of entries made by each mouse in the artificial plant treatment group into open arm-01 and open arm-02 and number and percentage of times a mouse reached the end open arm-01 and open arm-02

Artificial Plant Treatment						
	Number of entries	Number of entries	Entries reached to the end	Entries reached to the end	% of entries reached to the end	% of entries reached to the end
Mice No.	Open arm-01	Open arm-02	Open arm-01	Open arm-02	Open arm-01	Open arm-02
1	4	6	2	2	20	20
2	3	3	0	1	0	16.67
3	4	4	5	4	62.5	50
4	1	0	0	0	0	0
5	5	5	5	4	50	40
6	5	6	2	3	18.12	27.23
7	4	1	1	0	20	0
8	4	4	2	3	25	37.5
9	3	2	2	2	40	40
10	5	2	2	0	28.53	0
11	8	3	5	2	45.45	18.12
12	4	2	2	1	33.34	16.67
13	3	2	2	0	40	0
14	2	3	2	2	40	40
15	4	4	2	2	25	25
16	3	3	3	2	50	33.34
17	4	6	2	2	20	20
18	4	3	2	1	28.53	14.21
19	1	2	0	1	0	33.34
20	6	7	5	4	38.44	30.73

Table A18 Total number of entries made by each mouse in the live plant treatment group into open arm-01 and open arm-02 and number and percentage of times a mouse reached the end open arm-01 and open arm-02

Live plant treatment						
	Number of entries	Number of entries	Entries reached to the end	Entries reached to the end	% of entries reached to the end	% of entries reached to the end
Mice No.	Open arm-01	Open arm-02	Open arm-01	Open arm-02	Open arm-01	Open arm-02
1	0	0	0	0	0	0
2	9	9	5	6	27.78	33.34
3	2	3	2	2	40	40
4	6	9	4	8	26.67	53.34
5	4	1	3	1	60	20
6	5	2	5	2	71.47	28.53
7	3	4	2	2	28.53	28.53
8	2	4	1	1	16.67	16.67
9	3	2	0	1	0	20
10	5	6	1	1	9.09	9.09
11	4	3	1	1	14.21	14.21
12	4	3	2	1	28.53	14.21
13	5	4	3	1	33.34	11.11
14	3	1	1	0	25	0
15	4	2	1	0	16.67	0
16	6	6	4	4	33.34	33.34
17	8	9	4	3	23.51	17.66
18	3	3	2	2	33.34	33.34
19	7	8	5	4	33.34	26.67
20	2	3	0	0	0	0