The Effects of Housing Conditions on Anxiety-Like Behavior

by

Jade Jiang

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Jade Jiang

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_____

Dr. Mark D. Womble, Committee Member Date

_____

Dr. Diana Fagan, Committee Member Date

_____

Dr. Peter J. Kasvinsky, Dean, School of Graduate Studies & Research Date

ABSTRACT

Pain is modulated by multidimensional components. It is no longer adequate for us only looking into nociceptive component. One factor that plays a critical role in pain modulation is anxiety. Previous clinical studies have showed that affective pain is strongly associated with anxiety. The current study was to investigate the effects of cage complexity and social interaction on anxiety level. Four housing conditions as treatment groups were arranged, 1) three rats were housed in the same cage with toys (S/E), 2) three rats were housed in the same without toys (S/NE), 3) one rat was housed individually with toys (NS/E), 4) one rat was housed individually without toys (NS/NE). The subjects' anxiety level were assessed by open field test and elevated plus-maze test. The results showed that there were no statistically significant differences among the treatment groups, but there was a clear consistent trend demonstrated both in open field test and elevated plus-maze test that following the baseline data collection, after the rats (NS/E,NS/NE) were housed individually for a week, the anxiogenic profile increased that was indicated by the decrease in the time spent in the center square in open field apparatus and the time spent in open arms in elevated plus-maze test. And also the locomotive activities decreased in the number of time of exploring the novel object and the number of times of rearing events in open field apparatus. Isolation housing condition has been considered as a stress to organism. We speculated that isolation condition induced deregulation of HPA axis, it led to alteration of anxiety level.

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CHAPTER 1

I. Introduction

A. Perspectives on Pain

Nearly one in five Americans suffer from chronic pain and most claim that the pain is severe enough to interfere with their daily lives. With this increased recognition of pain and its impact on our society, many researchers have been drawn to the study of pain. Gaining a better understanding of pain mechanisms will provide more effective treatment to those who suffer from chronic pain conditions.

Pain is defined by the International Association for the Study of Pain (1994) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It serves as a protective mechanism for the body, producing a rapid reaction to noxious stimuli. Pain is a complex, sensory experience with subjective components including unpleasantness, displeasure, suffering and escape. From an evolutionary perspective, pain is believed to be the oldest sensation and there has been a debate during the last 100 years in terms of the biological significance of pain. One view proposes that pain is a sense similar to audition, olfaction, and vision, a component of the sensory repertoire of most animals (Basbaum and Bushnell, 2008). The other view is that unlike other senses, pain is a trigger of emotional states, a behavioral drive, and a highly effective leaning tool (Basbaum and Bushnell, 2008).

It is undeniable that pain is a very complex perception for scientists to examine not only due to the physiological qualities, but also the psychological and emotional components. The most modern view of pain is that it is a biobehavioral experience

associated with multiple components (Moore, 2009). One of the prominent components of pain is nociception, the sensory transmission of noxious information to the brain. Previously the term "pain" was often, mistakenly, used interchangeably with the term "nociception." The distinguishing factor is that nociception is purely a sensory quality. The other elements which are included in pain are affective, psychological, social and environmental elements. All of the components of pain are classified into three categories, such as sensory, affective and cognitive qualities (Nicolson, 2009), that interact with each other in the process of pain.

In 1894 Von Frey was the first to link pain to fine nerve terminals in the skin (Basbaum and Bushnell, 2008). The fine nerve endings of myelinated or unmyelinated afferents are called nociceptors since they are activated by noxious or nociceptive stimuli. By the 19thcentury, with increased evidence, the view of pain as a sense with a distinct physiological basis was overwhelmingly favored. Studies revealed that there were neurons in the spinal cord and brain driven exclusively by nociceptive stimuli. The noxious stimulus is converted to action potentials and this electrical signal is transmitted to the spinal cord via the Aδ or C afferent nerve fibers. At the dorsal horn of the spinal cord the first synapse occurs, and this is a site where the nociceptive information is highly modulated. Most of the nociceptive neurons' axons decussate at the level of the spinal cord entry and ascend to the brain via the spinothalamic tract. At the thalamus, the second synapse occurs and noxious information reaches the post-central gyrus of the parietal lobe of the cerebrum, an area known as the somatosensory cortex (Fig.1).

By the 1990s, there had been a significant increase in the number of studies conducted on the affective dimension of pain. The affective dimension of pain includes

feelings of unpleasantness and emotions. This strongly correlates to the environmental context in which the noxious stimuli are presented and not to the intensity of the nociceptive stimuli. In terms of pain pathways, pain has a relative separation of cerebral structures that are involved in the affective aspects of the pain experience as compared to the nociceptive components (Rainville, 2002).

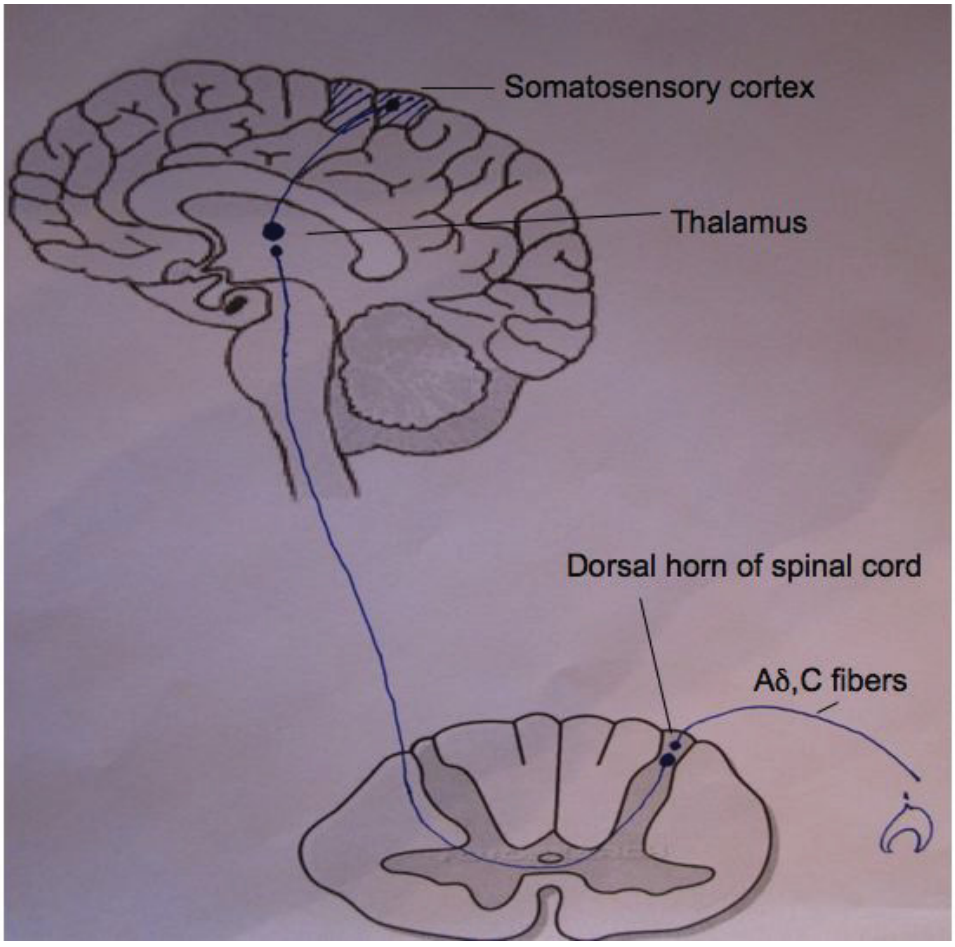

Figure 1. Spinothalamic pain pathway

The information from nociceptors not only projects to the somatosensory cortex, but also projects collaterally to the prefrontal lobes, amygadala, and anterior cingulate cortex, which are all substructures of the limbic system. These structures form the anatomical

basis for the emotional components of pain. Interestingly, these substructures are also brain areas associated with depression and anxiety disorders. Researchers estimate that 20% to 50% of patients with chronic pain also have depression and anxiety (Nicolson, 2009). It seems convincing that a strong link between affective pain and anxiety/depression disorders exists. This may suggest that pain treatment can be optimized by simultaneously treating depression and anxiety disorders.

B. Nociception

1. Nociceptor

The physiological component of pain, nociception, has been widely studied. Nociception includes the processes from transduction of the noxious stimulus into an action potential, to arrival of the impulse at the somatosensory cortex. The nociceptors are specifically designed to process the sensation of pain by preferentially responding to intense, noxious stimuli with a high threshold. The function of nociceptors is to inform the central nervous system (CNS) of high intensity stimuli which may cause tissue damage. Nociceptors are distinguished from innocuous touch receptors by their relatively high threshold for activation (McMahon and Koltzenburg, 2005). Further, nociceptors are the peripheral terminal of either thinly myelinated Aδ fibers or nonmyelinated C fibers. Aδ fibers (fast pain pathway) are responsive to noxious temperature stimuli with relative high velocity, 5-30 m/sec. C fibers (slow pain pathway) are polymodal responding to temperature, mechanical and chemical stimuli with low velocity 0.5-2m/sec. They are classified mainly into cutaneous nociceptors and deep tissue nociceptors (Basbaum and Bushnell, 2008). Cutaneous nociceptors respond to noxious stimuli including mechanical, thermal and chemical (algesic molecules) stimuli, and transducing cutaneous pain. Deep

tissue nociceptors found in muscles, joints, and viscera which respond exertion or injury by muscle nociceptors, inflammation by joint nociceptors and overdistention, ischemia, inflammation in viscera respectively (McMahon and Koltzenburg, 2005).

Nociception begins with depolarization of nociceptors by noxious stimuli. A noxious stimulus will elicit the opening of voltage-gated sodium ion (Na^+) channels. In order to trigger the action potential, the nocicpetor has to be depolarized to a threshold potential (-55mV) by Na^+ influx. Resting membrane potential is usually -70mV, which is established by ionic concentration gradients. The sodium/potassium (Na^+/K^+)-ATPase pump moves three Na^+ ions to the outside of the cell and two K^+ ions to the inside of the cell, which maintains resting membrane potential. This action potential process is a all or none response. As long as the depolarization by Na^+ channel influx reaches the threshold potential, the transduction of pain is achieved at a functional meaning that completes the pain received in the cerebral cortex (Pace, 2006). In the other words, Pain is not able to be perceived in the brain until the action potential is achieved that is caused by the noxious stimulus triggering the free nerve ending to reach the threshold.

Transient receptor potential vanilloid (TRPV) channels are embedded in the plasma membrane of the nociceptor. This is a family of transient receptor potential ion channels that play a role as a transducer in the nociceptive neurons. Noxious chemicals, thermal, and mechanical stimuli are transduced by TRPV into membrane depolarization expressed as an electrical signal (Hunt and Koltzenburg 2005). These are Na^+ channels that produce excitatory postsynaptic potentials (EPSPs). Certain agents such as protons and capsaicin act directly on TRPV, and are regulated by the opening of TRPV channels permeable to Na^+ and/or calcium ion, and lead to depolarization of the nociceptive

neurons. TRPV1, a main member of TRPV, is identified as a transducer for a wide variety of exogenous and endogenous physical and chemical stimuli. TRPV2 has been discovered to be activated by high temperature, specifically when it is above 52^0 C (Pace, 2006).

2. Entry into the CNS

Specialized afferent neurons (Aδ, C) enter the spinal cord via dorsal roots. Some fibers synapse at the spinal cord level of entry, and some ascend or descend several segments decussating in the anterior white commissure. The nociceptive information is carried by primary afferent fibers, specifically Aδ fibers transmitting fast pain, that synapse on second-order neurons in the lamina I marginal nucleus of the dorsal horn (Hunt and Koltzenburg 2005). For the slow pain pathway, afferent fibers primarily are C fibers that carry nociceptive information that synapse onto second order neurons in the lamina II sunstantia gelatinosa, which are referred to as the superficial part of the dorsal horn, and also synapse on lamina V nucleus proprius, located in the deep part of the dorsal horn. Aδ fibers terminate within laminas I and C fibers terminate in laminas II and V. As the lamina I marginal nucleus plays a key role in the modulation of pain, most pain studies focus on this specific lamina (Basbaum and Bushnell, 2008).

3. Anterolateral System Pain Pathways

The nociceptive signal from dorsal horn neurons is transmitted via ascending pathways termed the spinothalamic tract located in the anterolateral quadrant of the spinal cord. Spinothalamocortical pathways convey nociceptive signals to make the final synapses on multiple areas of the brain: the primary and secondary somatosensory cortices, the insula, and the anterior cingulate cortex. Most of the cells project to the

contralateral thalamus where the second synapse occurs (McMahon and Koltzenburg, 2005). Laterally spinothalamic neurons are more likely to be situated in lamina I and V which is a fast pain pathway conveyed by Aδ fibers, whereas medially projecting spinothalamic neurons are more likely to be situated in lamina II the deep dorsal horn and in the ventral horn and they are primarily conveyed by C fibers to transmit the slow pain (Basbaum and Bushnell, 2008). Spinothalamic axons in the anterolateral quadrant of the spinal cord are arranged somatotopically which means at the spinal cord level, each anatomical cross section area consisted of axons corresponds to the certain parts of the body.

C. The Pathway for Affective Pain

In regards to the emotional component of pain, it is conveyed through a separate pathway, termed the affective pain pathway. The differentiation of the affective pain pathway from the sensory pathway begins at the dorsal horn of the spinal cord. The sensory information follows the classical spinothalamic tract pathway. In parallel, there are additional pathways which contribute to the emotional and cognitive aspects of pain, termed divergent pathways, including the paleospinothalamic, spinomesencephalic, and spinoreticular tracts. These pathways synapse on neurons located in the limbic system or cotico-limbic system. The limbic system is used as a collective term for various brain structures including the hippocampus, parahippocampal gyrus, amygdala, and cingulate gyrus (anterior cingular cortex, posterior cingular cortex). All of these structures are associated with processing emotion and cognitive information. The affective part of pain information follows through divergent pathways to synapse on the neurons in limbic system. Two pathways are particularly relevant, the cortico-limbic pathway and the direct

limbic pathway. Divergent fibers from part of the spinal cord go through the paleospinothalamic tract to the dorsocaudal medulla oblongata, the subnucleus reticularis dorsalis, then to the ventromedian nucleus of the thalamus, and finally to the dorsolateral frontal lobes; that is classified as cortico-limbic pathway. The other fibers follow the spinomesencephalic tract or spinoreticular tract to go to the parabrachial nucleus and subsequently to the hypothalamus and amygdala; this is classified as the direct limbic pathway. These two affective pathways both converge on the same anterior cingulate cortex and subcortical structures. As we see the pain information is not only to reach the somatosensory areas of the brain, but also it projects to the limbic system that is responsible to process the emotional component of the pain.

D. Link between Pain and Anxiety

Up to now, the sensory component of pain related to nociception has been more widely studied and better understood than the affective component. Since the modern view of pain is accepted as a biobehavioral process resulting from a complex interaction among the sensory, affective, cognitive and behavior components, a new challenge has arisen and prompted researchers to explore the affective aspects of pain with regards to the psychological and emotional qualities, which may be the most relevant component of human pain.

Evidence from recent functional brain imaging studies has demonstrated the anatomical link between pain and anxiety. Tolle (1999) reported that subject's ratings of pain unpleasantness were correlated with activity in the caudal anterior cingular cortex, a primary limbic structure in the regulation of anxiety and depression. Rainville (2002) discovered that activity within the anterior cingulate cortex and the other substructures of

the limbic system may contribute to the modulation of affective pain. An experiment done by Rainville (1997) which provided convincing evidence of an additional pathway involved in substructures of the limbic system responsible for the emotional aspect of the pain experience. Positron Emission Tomography (PET) scan was used to detect the activity in different areas of the brain in two groups of human volunteers. In group one, the subject's left hand was immersed in hot water maintained at constant, noxious temperature at 47^{o}C (under the modulation of intensity of nociception). Group two subjects were under varied level of hypnotic suggestions (under the modulation of pain unpleasantness). Hypnosis was used as a cognitive tool to alter the unpleasantness component of pain. Results showed that the manipulation of pain unpleasantness produced significant brain activity changes in the anterior cingulate cortex, specifically the high level of unpleasantness induced high activity of the anterior cingulate cortex, but no changes in the primary somatosensory cortex. In contrast, the manipulation of nociceptive intensity from the sensory aspect produced changes mainly in somatosensory cortex. Also, Neugebauer et al. demonstrated a relationship between persistent pain and the activity level of amygdala (McMahon and Koltzenburg, 2005). The amygdala is the structure related to a variety of psychological states such as anxiety, depression, and fear. Interestingly, from an anatomical aspect, both the affective component of pain and anxiety/depression are related to the same anatomical basis of the limbic system. Kenshalo discovered clinically the activation of structures such as the insula and the anterior cingulate cortex may enhance the affective aspect of the pain experience (McMahon and Koltzenburg, 2005). In a reverse way, research showed that surgical lesions of the cingulate cortex demonstrated alleviation of emotional pain, but not the

sensory component of chronic pain. Patients who were suffering from anxiety and depression showed substantial relief after they had cingulotomies (McMahon and Koltzenburg, 2005). And also Katja (2010) reported his research results derived from a large sample in Germany (N=7,124) that pain was strongly associated with anxiety disorders and seemed to be of equal or greater strength compared to the pain-depression association. Adrienne (2008) discovered that treating anxiety and depression may, in turn, reduce the experience of pain. This study suggests that if we treat anxiety, simultaneously the perception of pain would be reduced. This provided the initial basis for the current study. An ultimate question of interest is in order to treat pain effectively, if anxiety is treated, can this reduce the perception of pain? This is a broad question, but the current study will provide an initial step to work toward the answer.

Previous work in this laboratory has shown housing environment affects nociceptive pain (Tall, 2009). Based on the link between pain and anxiety, the current study will determine if cage complexity and social interaction affect anxiety-like behaviors in rats. If the results are positive, this will provide more evidence of the connection between pain and anxiety. Specifically, this study's objectives include: 1) How social interaction without cage enrichment affects the level of anxiety; 2) How isolation without cage enrichment affects the level of anxiety; 3) How the combination of social interaction and cage enrichment affects the level of anxiety; 4) How isolation with cage enrichment affects the level of anxiety.

CHAPTER 2

II. Materials & Methods

A. Animals

The subjects were male Sprague-Dawley rats (22-24 days at the time of arrival; n=24) obtained from Charles-Rivers, Laboratories in Wilmington, MA. All animals were naïve to the experimenters and had not participated in any previous treatments. Initially rats were housed three per cage in polycarbonate cages (20 inches long, × 16 inches wide, × 8 inches high) with aspen chips and cob-of-corn bedding in the animal care facility. The facility was maintained on a 12/12h light/dark cycle, with lights off at 10:00 A.M. and lights on at 10:00 P.M. The animal facility temperature was maintained at 21 ± 1^{0}C and humidity at 51%. Tap water and rodent chow (Proab RMH, MO) were provided *ad libitum*. The rats were allowed to acclimate to the animal care facility for one week prior to any type of intervention. During the second week, all rats were acclimated to the investigators and behavioral research laboratory for three consecutive sessions. Each session each rat was acclimated with the investigator for 5 minutes. All experimental testing was approved by the Institutional Animal Care and Use Committee at Youngstown State University, and followed the ethical guidelines of the International Association for the Study of Pain. Experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH publications No. 80-23, revised 1996).

B. Housing Conditions

The rats were randomly assigned to a housing treatment group by varying social and environmental enrichment factors. Four conditions were examined: 1) In the social enriched condition (S/E), three rats were housed in the same cage containing various toys; 2) In the social non-enriched condition (S/NE), three rats were housed in a standard cage without toys; 3) In the non-social enriched condition (NS/E), one rat was housed individually in a standard cage containing various toys; 4) In the non-socially non-enriched condition (NS/NE), one rat was housed in a standard cage without toys. The objects used to provide cage enrichment included Nylabones, polycarbonate tunnels, DNA flexer, Hol-ee mol-ee balls, Dental balls, crawl balls and Rodent Retreats (Bio-Serve, Frenchtown, NJ; Fig. 2) The purpose of these items was to increase the complexity for rats assigned to the environmental enrichment condition. The items were rotated with clean, novel items once per week.

C. Behavioral Testing

Baseline behavioral data were collected before rats were assigned to the housing treatment group. Before baseline data collection, all rats were housed in a group of three in a random fashion in a standard cage. The behavioral data was collected once per week for total of five weeks. The investigators performed the open field test first and then the elevated plus-maze test between 11:00 AM and 5:00 PM, during the rat's dark phase. Both tests are useful tools to evaluate the level of anxiety by quantitively measuring the rats' spontaneous behavior. Previous work has shown that the elevated plus-maze test has been used in preclinical trials for approximately a decade and validated as a tool to identify the anxiolytic effects resulting from drugs and environmental factors. It is worth mentioning that there are several advantages to utilizing this equipment, such as

measuring spontaneous behaviors, removing the need for nutritional deprivation, avoiding the use of aversive stimulation, and it is relatively inexpensive (Darwish, 2001). The behavioral data were recorded using a video camera (HandyCam Vision, Video Hi8) mounted on the ceiling, and files were digitized by Pinnacle software. This set up allowed the investigators to perform real time data analysis or to view the files later.

Figure 2: Home Cage Enrichment. Rats assigned to the enriched condition (right cage) have the inclusion of items shown in the picture in contrast to the rats assigned to non-enriched cage without objects (left cage).

D. Assessment of Behavioral Responses in an Open Field

The equipment for the open field test was made of an opaque black Plexiglas chamber (length=120 cm, width=80 cm, wall height=40 cm). The chamber consisted of 24 squares, with 16 outer squares and 8 inner squares (Fig. 3). Exploratory behaviors in the chamber were recorded for 5 minutes from each subject. Alcohol (70%) was used to thoroughly clean the chamber at the end of each trial to eliminate the olfactory cues for subsequent test subjects. A novel object was placed at the center of the chamber. The objects were rotated each week allowing exposure to a novel object. At the start, each rat was placed into the right corner of the chamber facing the center of the open field. The following parameters were recorded by the video camera mounted on the ceiling: time in the center squares, the number of rearing events, the number of urination events, the number of defecation events, and the number of times exploring the novel object. These variables were served as measures of anxiety-related behaviors.

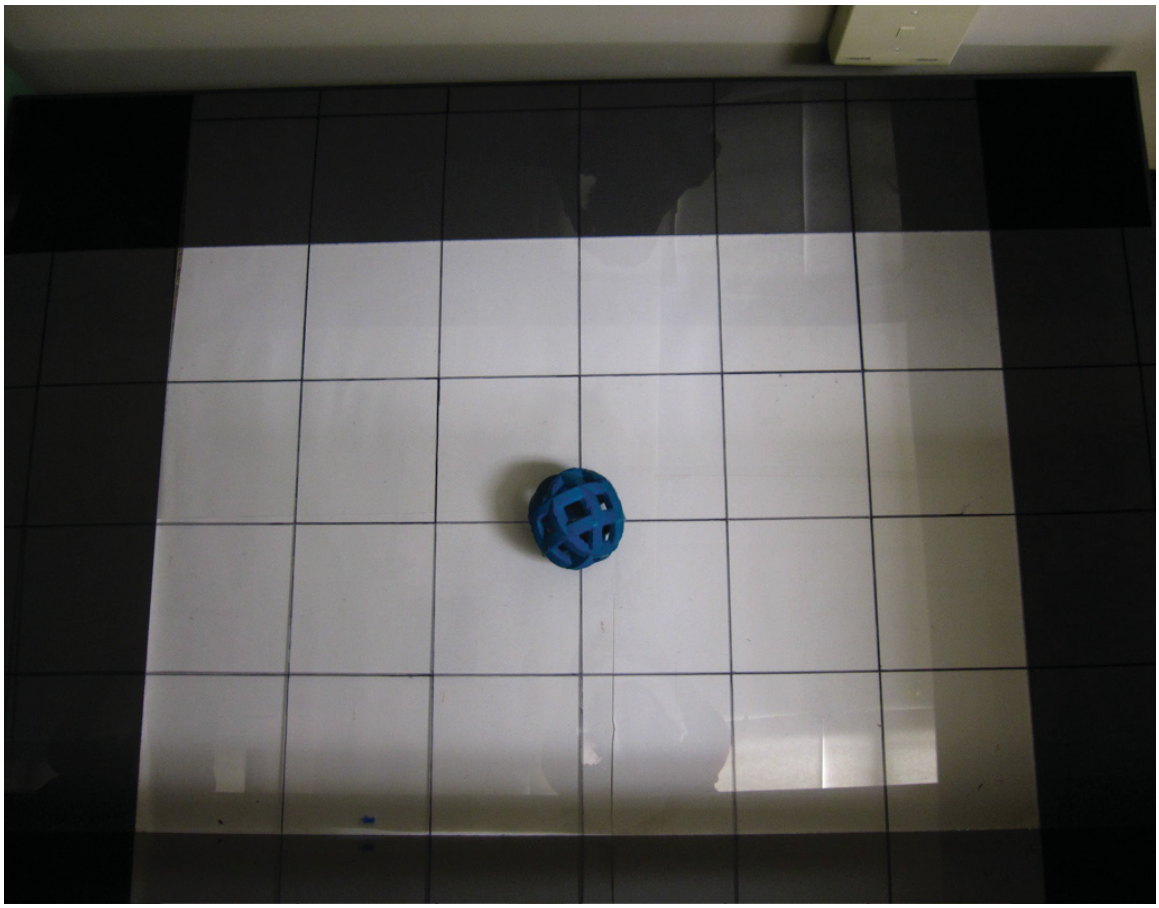

Figure 3. Behavioral Assessment in an Open Field Test. Each subject's exploratory behavior was measured in the open field. The multiple parameters were measured including the number of rearing events, the number of urination events, the number of defecation events and the number of exploring the novel object. The size of opaque black Plexiglas chamber (length120cm x width 80cm x height 40cm).

E. Assessment of Behavioral Response in Elevated Plus-Maze

The elevated plus-maze was also used to measure anxiety-like behaviors and was made of two open arms (45x10 cm) and two closed arms (45x10x30 cm), standing 52 cm off the floor. It was made of opaque Plexiglas walls sitting on four cylinder shape stands (Fig. 4) The rats' exploratory behavior in the maze was recoded by a video camera

mounted 145 cm above the maze. At the start, each rat was placed into the center of a Plexiglas elevated plus-maze facing an open arm and each trial lasted 5 minutes. At the end of each trial, the maze was thoroughly cleaned with 70% alcohol and dried to remove olfactory cues that might affect the behavior of the subsequent trials. The following parameters were recorded: the number of open arms entries, the number of closed arms entries, time spent in the open arms, and the number of rearing events. The criterion for open arm entry was at least 70% of the rat's body had to enter the open arm; the criterion for closed arm entry was at least 70% of rat's body had to enter the closed arm. The rearing behavior was defined as both front paws lifted above the floor and all weight on the hint paws. Again, all these variables served to measure anxiety-related behavior. The rats were transported from the animal facility to the behavioral neuroscience lab at 10:30 am, and subsequently the open field test and elevated plus-maze test were implemented by the investigators. There was no gap between the two tests.

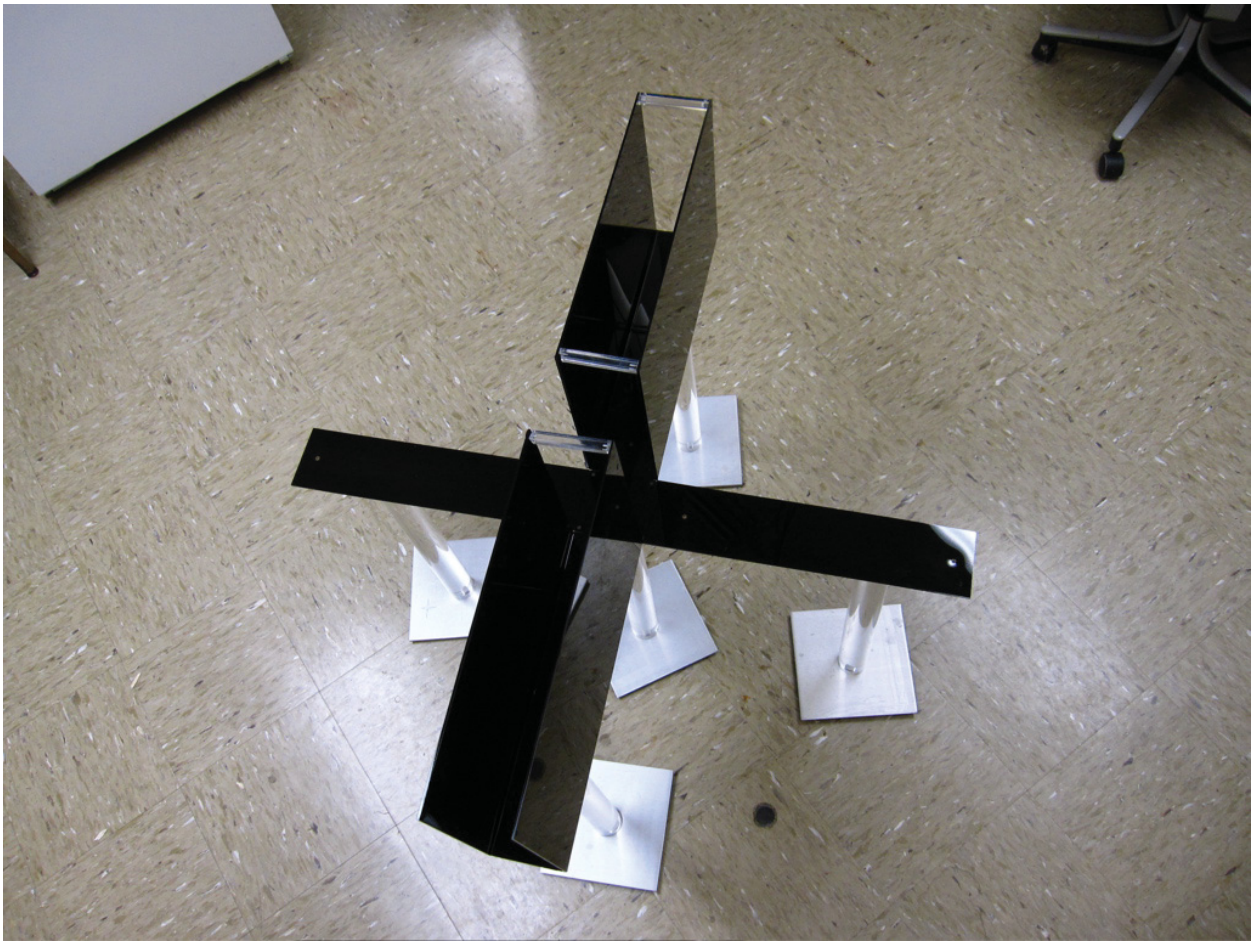

Figure 4. Behavioral Assessment in Elevated Plus Maze Test. The multiple parameters were measured, the number of open arms entries, the number of closed arms entries, time spent in the open arms and the number of rearing events. Two open arms (45x10 cm) and two closed arms (45x10x30 cm), standing 52 cm off the floor.

F. Statistics

For each behavioral measure, a repeated measures, two-way analysis of variance (ANOVA) was conducted to determine if there was a significant difference among the treatment groups over time (SPSS, Version 13.0). A p-value ≤ 0.05 was considered statistically significant. The independent (grouping) variables were the four housing

conditions (S/E, S/NE, NS/E, NE, NE). The dependent variables were the anxiety-like behaviors from the open field test and the elevated plus-maze. All data are expressed as the mean ± S.D.

CHAPTER 3

III. Results

A. ANOVA for Open Field Apparatus

A repeated measures, two-way ANOVA was conducted to determine if there was a significant difference among the four housing conditions. The dependent variables measured included multiple parameters such as the time spent in the center squares, the number of times exploring novel object, the number of rearing events, the number of urination events, the number of fecal pellets. All results are presented as the mean ± S.D. P-values among housing conditions and among the weeks were computed (SPSS version 13.0). A P-value of ≤0.05 was considered statistically significant.

1. **The Time Spent in the Center Squares of the Open Field Apparatus**

The time spent in the center squares in the open field is illustrated in Figure 5. The anxiety-like behavior measured by the time spent in the center squares is the indication of the level of anxiety. The longer time spent in the center squares, the less anxious is indicated. The results of the ANOVA showed that there were no significant differences among the four housing conditions (P=0.28), and no significant differences among the test weeks (P=0.23). There was a noticeable trend toward spending less time in the center squares in the non-social groups (NS/E, NS/NE) during the first week of behavioral data collection. The results showed that there was a trend such that an inverse relationship was seen between social housing and an anxiogenic effect in the open field test. In addition, the data from week 2 through week 5 showed a habituation effect that was demonstrated by no consistent change in the time spent in the center squares.

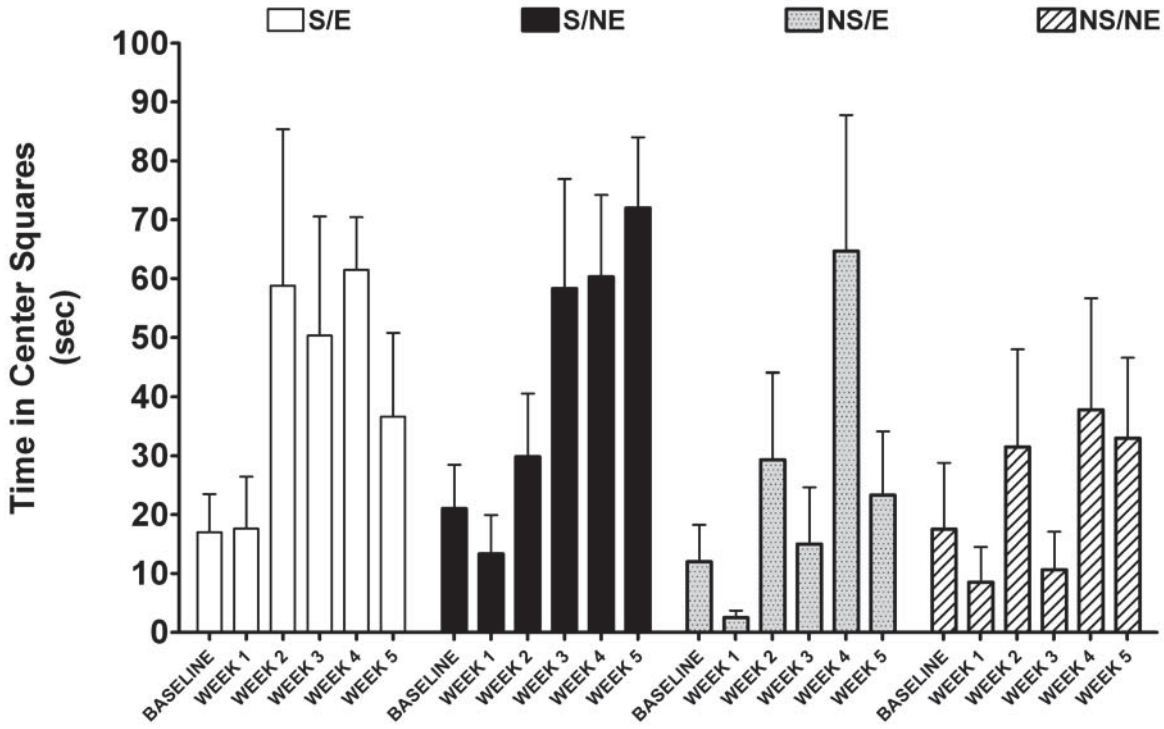

Figure 5: The Time Spent in the Center Squares in the Open Field Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the time spent in the center squares following repeated measurement for baseline, week1, week2, week3, week4, week5 (P1= 0.28, P2=0.23, P1-among 4 housing conditions, P2- among testing weeks). At least 70% of the rat's body entering the center squares is considered as in the center squares when we measured the behavior parameter "time spent in the center squares." S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6) ,NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

2. The Number of Events Exploring a Novel Object in the Open Field Apparatus

The number of events exploring a novel object in the open field test is illustrated in Figure 6. The number of events exploring a novel object were measured as anxiety-like behavior. The higher number of exploring a novel object, the less anxious is indicated. The results of the ANOVA showed that there were no significant differences among four housing conditions (P=0.09), and no significant differences among the testing weeks (P=0.36). There was a noticeable trend in non-social groups (NS/E, NS/NE) during the first week of behavioral data collection, following baseline. The number of events exploring the novel object in those groups decreased after a week of living in isolated housing conditions. The data showed the same pattern as the time spent in the center squares which indicated an inverse relationship between social housing and an anxiogenic effect. In social and enriched housing groups, at week 1, there was a noticeable increase in the number of occurrences of exploring the novel object. In addition, the data from week 2 through week 5 showed a habituation effect that was demonstrated by no consistent change in exploratory behavior.

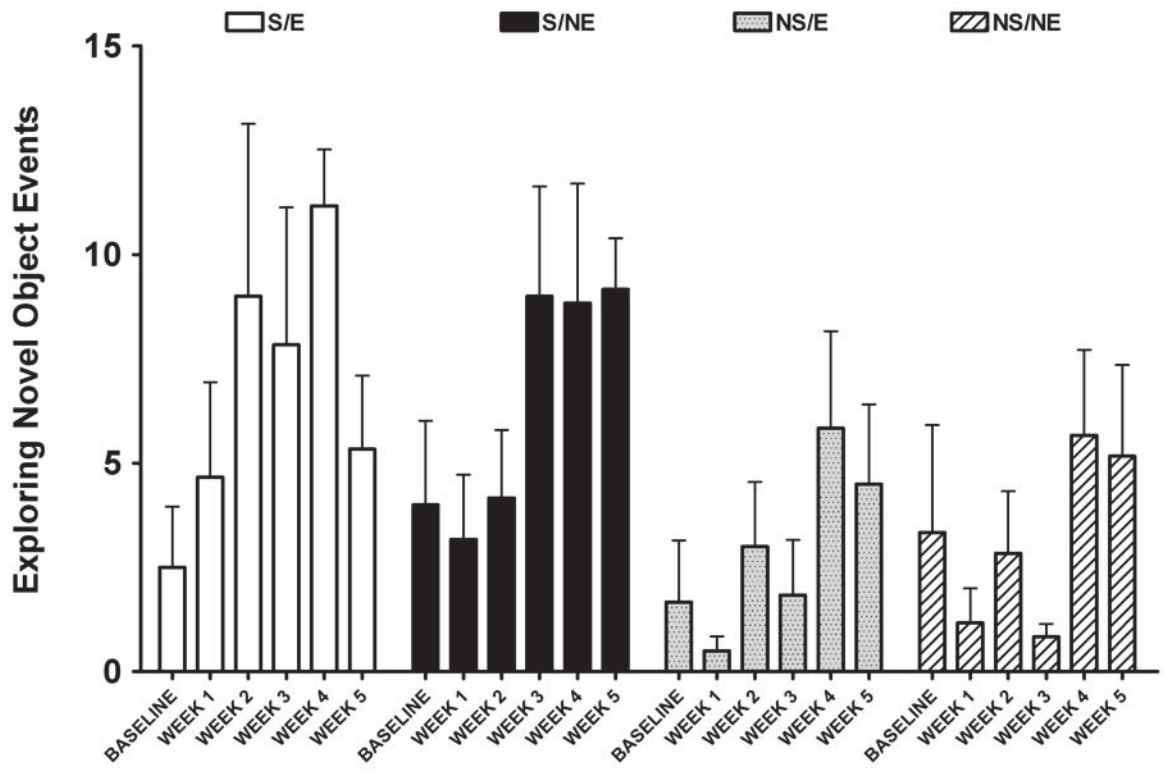

Figure 6: The Number of Events Exploring the Novel Object in the Open Field Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the number of events exploring the novel object following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.09, P2=0.36, P1-among 4 housing conditions, P2-among testing Weeks). Exploring a novel object event is defined as when the rat's nose or it's head touching the novel object. S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

3. The Number of Rearing Events in the Open Field Apparatus

The number of rearing events in the open field test is illustrated in Figure 7. The number of rearing events were measured as anxiety-like behavior. The higher number of

rearing events, the less anxious is indicated. The results of the ANOVA showed that there were no significant differences among four housing conditions (P=0.31), and no significant differences among the testing weeks (P=0.15). There was a noticeable trend toward the number of rearing events decreased in nonsocial/enriched (NS/E) group , nonsocial/non-enriched (NS/NE) group and social non-enrichment (S/NE) group during the first week of behavioral data collection, following baseline. The data of anxiety-like behavior, the number of rearing events supported the same trend as those data of the time spent in the center squares and the number of exploring the novel object. They all showed a trend such that there was an inverse relationship was seen between social housing and anxiogenic effect. In addition, the data from week 2 through week 5 showed a habituation effect that was demonstrated by no consistent change in the number of rearing events in open field test.

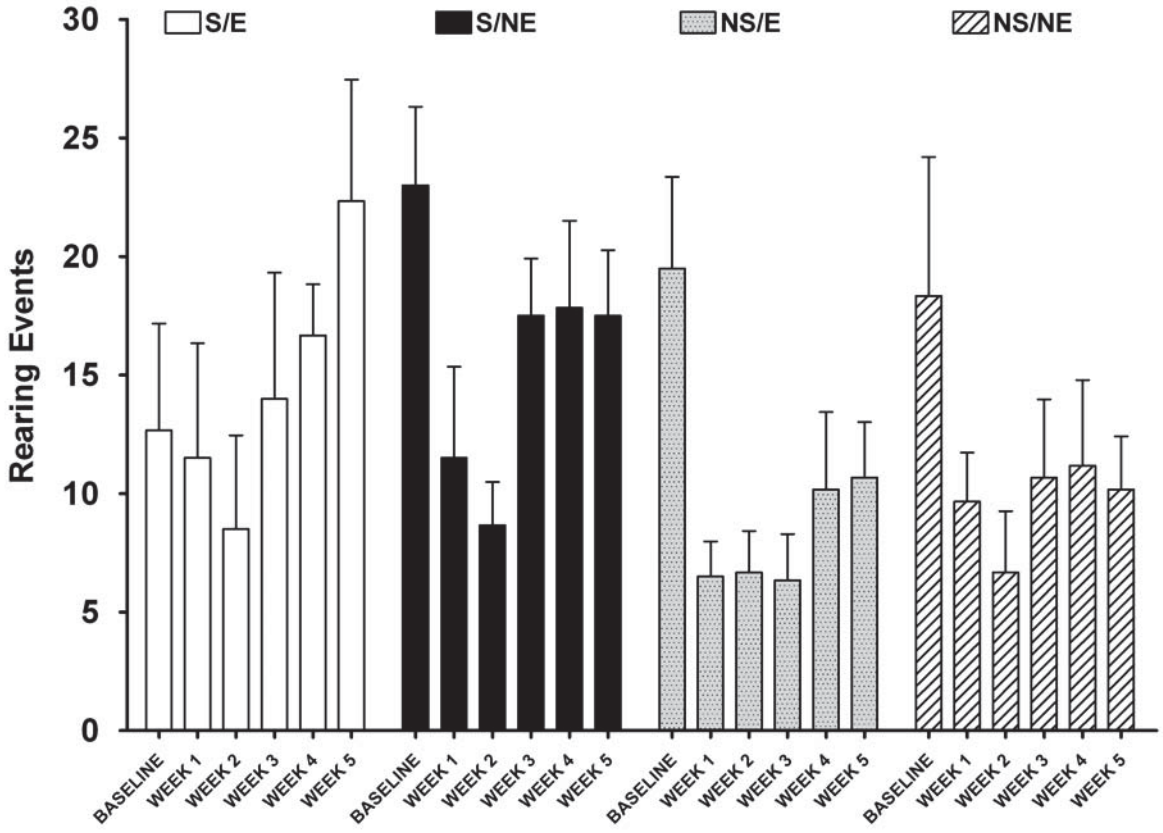

Figure 7: The Number of Rearing Events in the Open Field Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the number of rearing events following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.31, P2=0.15, P1-among 4 housing conditions, P2-among testing Weeks). Rearing event is defined as both front paws lifted up above the floor. S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

4. The Number of Urination events in the Open Field Apparatus

The number of urination events in the open field test is illustrated in Figure 8. The number of urination events were measured as anxiety-like behavior. The higher number

of urination events, the more anxious is indicated. The results of the ANOVA showed that there were no significant differences among the four housing conditions (P=0.22), and no significant differences among the testing weeks (P=0.33). There was a slight decrease in the number of urination in both nonsocial enriched group (NS/E) and nonsocial non-enriched group (NS/NE) during the first Week behavioral data collection, following the Baseline. In addition, the data from week 2 through week 5 showed habituation effect that was demonstrated by no consistent change in the number of urination events in the open field test.

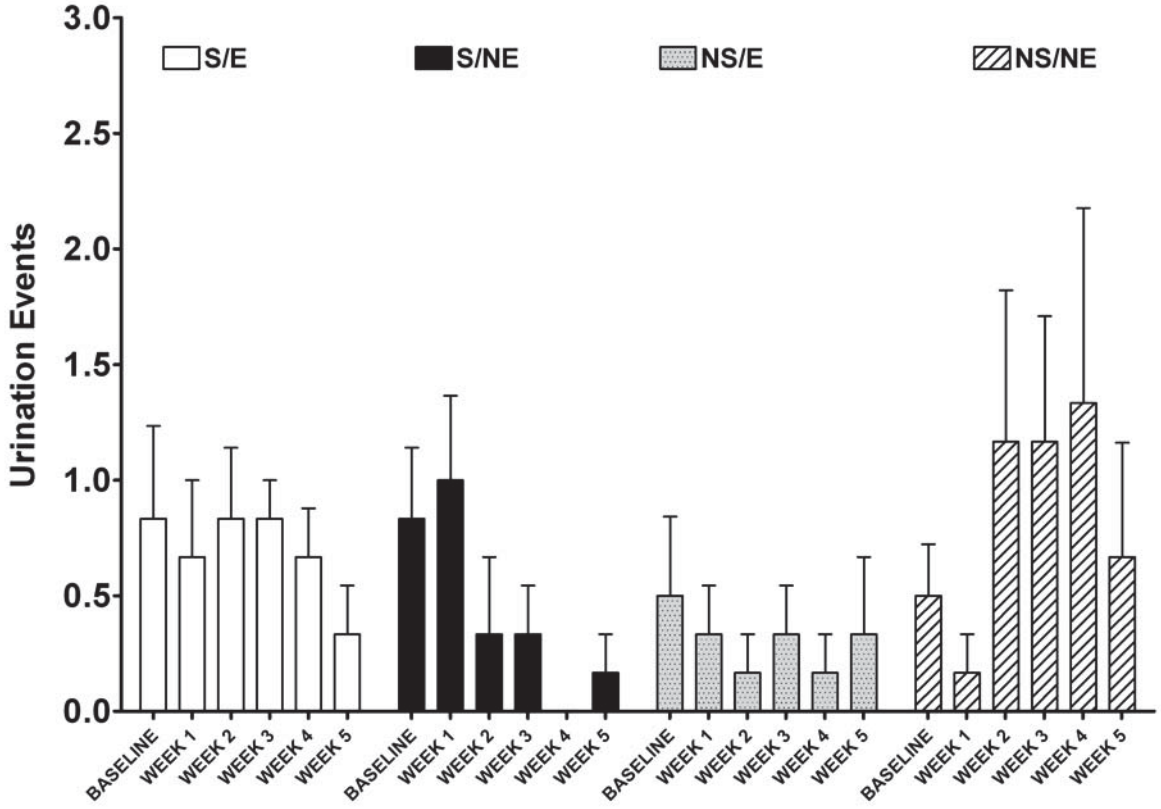

Figure 8: The Number of Urination Events in the Open Field Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the number of urination events following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.22, P2=0.33). S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

5. The Number of Fecal Pellets in the Open Field Test

The number of fecal pellets in the open field apparatus is illustrated in Figure 9. The results of the ANOVA showed that there were no significant differences among four housing conditions (P=0.81), and no significant differences among the testing weeks (P=0.84).There was no any pattern or trend that was noticed. In addition, the data from week 2 to week 5 showed habituation effect that was demonstrated by no consistent change in the number of fecal pellets.

B. ANOVA for Elevated Plus-Maze Test

A repeated measures, two-way ANOVA was conducted to determine if there was a significant difference among the four housing conditions. The dependent variables measured included the time spent in the open arms, the number of rearing events and open arms entries in the elevated plus-maze. All results are presented as the mean ± S.D. P-values among housing conditions and among the weeks were computed (SPSS version 13.0). A P-value of ≤0.05 was considered statistically significant.

1. The Time Spent in the Open Arms in Elevated Plus-Maze Test

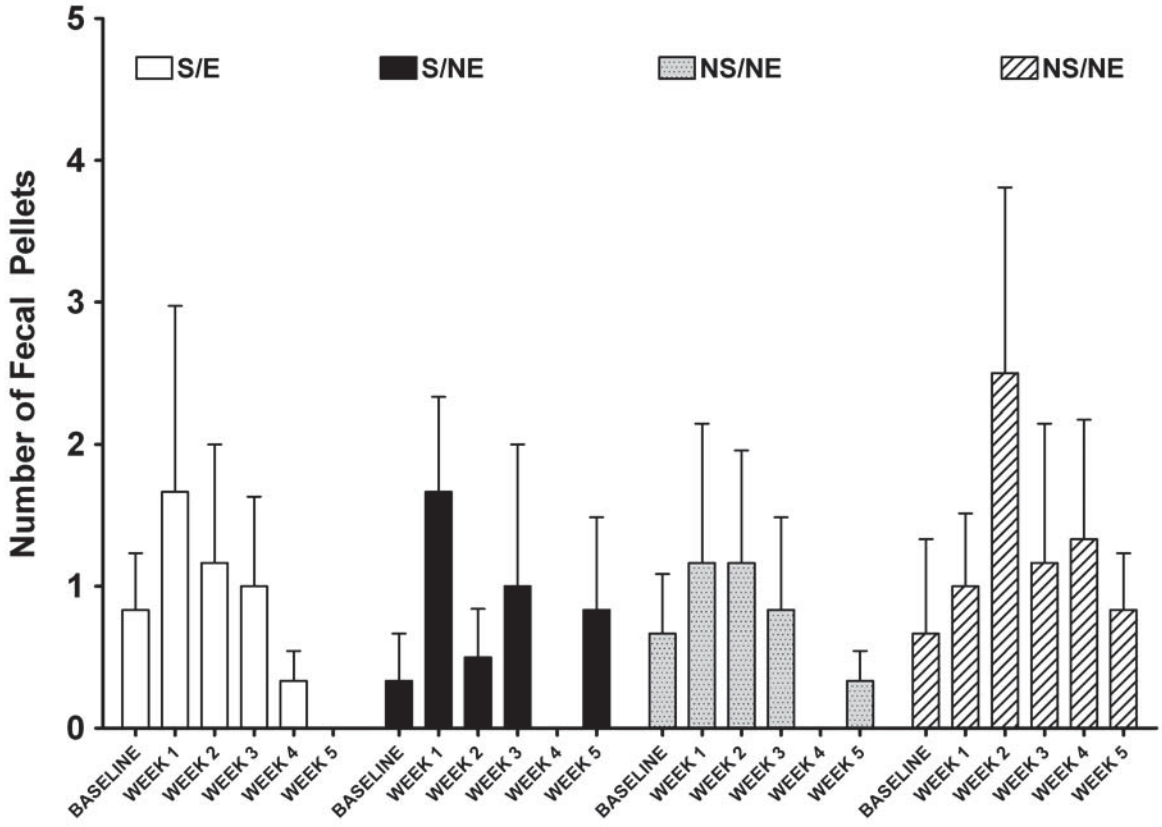

Figure 9: The Number of Fecal Pellets in the Open Field Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the number of fecal pellets following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.81, P2=0.84). S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6),

NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

The time spent in open arms in the elevated plus-maze is illustrated in Figure10. The anxiety-like behavior measured by the time spent in the open arms is the indication of the level of anxiety. The longer time spent in the open arms, the less anxious is

indicated. The results of the ANOVA showed that there were no significant differences among the four housing conditions (P=0.19), and no significant differences among the testing weeks (P=0.17). The data showed that there was a noticeable trend toward spending less time in the open arms in non-social groups (NS/E, NS/NE) during the first week behavioral of data collection. The results showed there was a trend such that an inverse relationship was seen between social housing and anxiogenic effect in the elevated plus- maze test that was in accordance with those data in the open field test. In addition, the data from week 2 through week 5 showed habituation effect that was demonstrated by no consistent change in the time spent in the open arms.

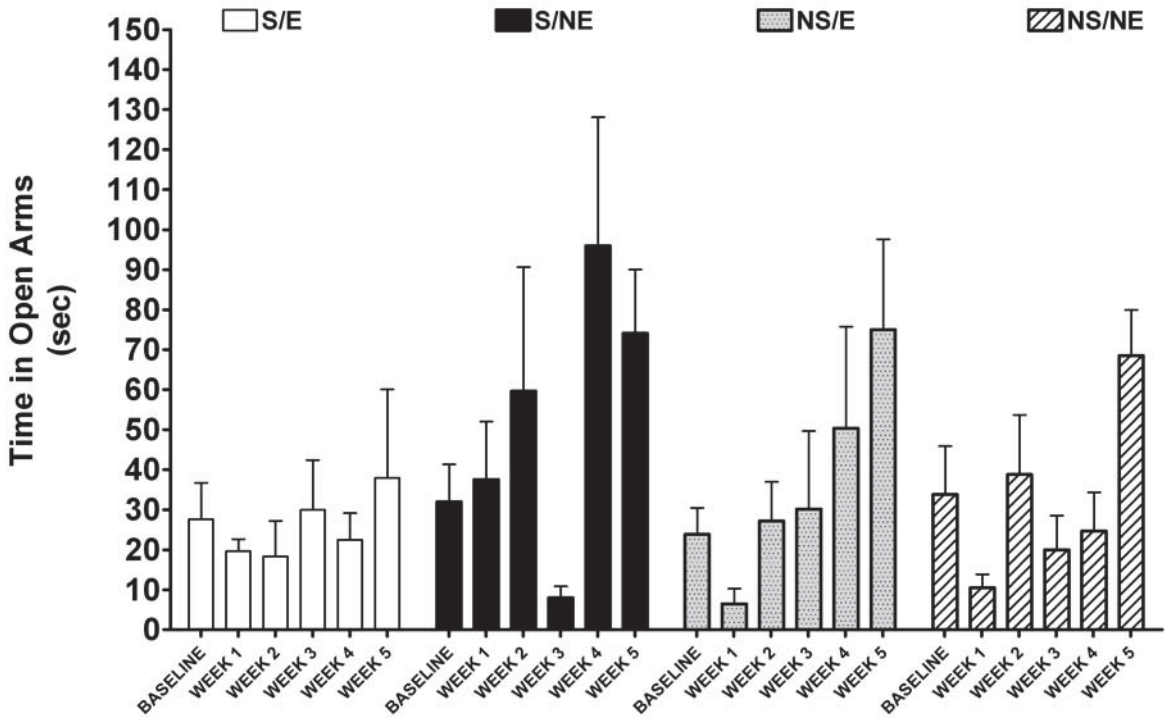

Figure 10: The Time Spent in the Open Arms in Elevated Plus-Maze Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the time spent in the open arms following repeated measurement for baseline, week 1, week 2, week 3, week

4, week 5 (P1= 0.19, P2=0.17). At least 70% rat's body entering the open arms considered as staying in the open arms when we measured the time spent in the open arms. S/E= Social and Enriched Group (n=6), S/NE= Social and Non Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

2. The Number of Rearing Events in Elevated Plus-Maze Test

The number of rearing events in the elevated plus-maze is illustrated in Figure 11. The anxiety-like behavior measured by the number of rearing events in elevated plus-maze is the indication of the level of anxiety. The higher number of rearing events, the less anxious is indicated. The results of the ANOVA showed that there was no significant difference among the four housing conditions (P=0.89), and no significant difference among the testing weeks (P=0.55). The data showed that there was a noticeable trend in non-social groups (NS/E, NS/NE) during the first week behavioral data of collection, following baseline. For those non-social groups the number of rearing events decreased after a week of living in isolated housing conditions. In addition, the data from week 2 through week 5 showed habituation effect that was demonstrated by no consistent change in the number of rearing events in the elevated plus-maze.

3. The Open Arms Entries in the Elevated Plus-Maze Test

The open arms entries in elevated plus-maze is illustrated in Figure 12. The anxiety-like behavior measured by the open arms entries in the elevated plus-maze is the indication of the level of anxiety. The higher number of open arms entries, the less anxious is indicated. The results of the ANOVA showed that there was no significant difference among the four housing conditions (P=0.33), and no significant difference

significance among the testing weeks (P=0.25). In addition, the data from week 2 through week 5 showed habituation effect that was demonstrated by no consistent change in the open arms entries in the elevated plus-maze.

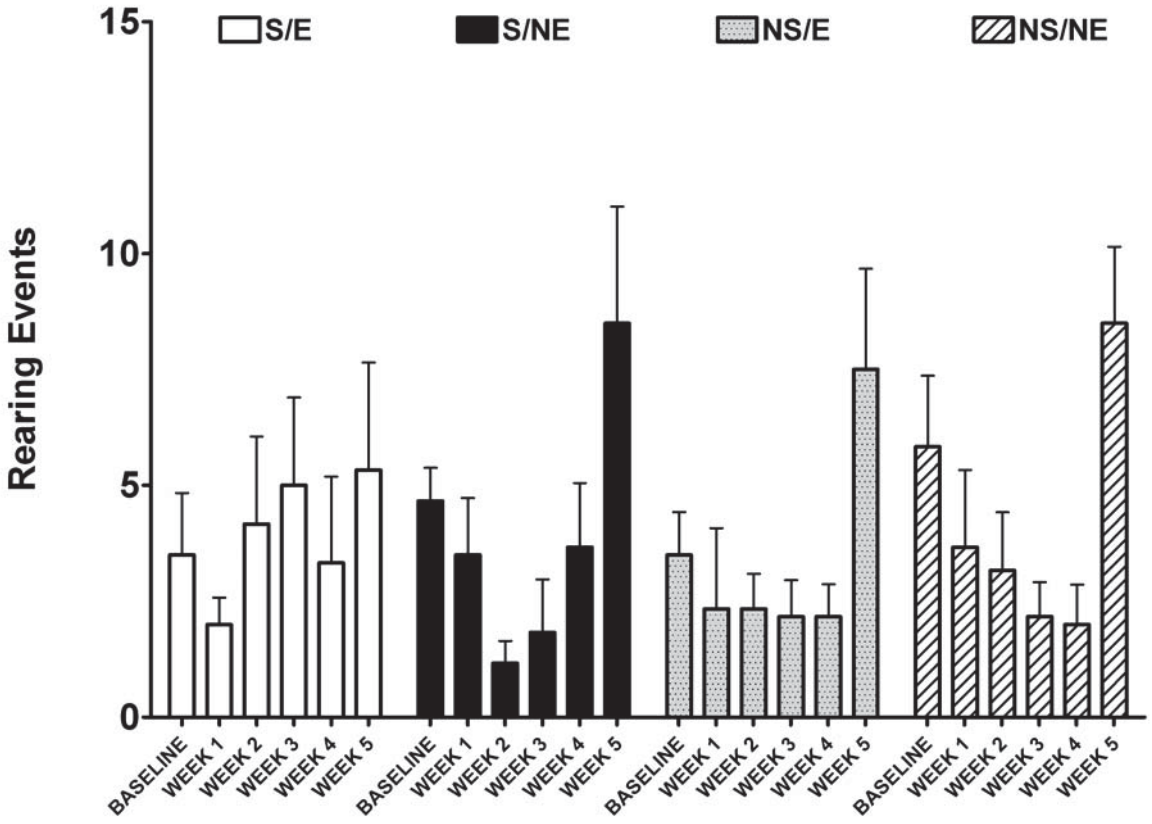

Figure 11: The Number of Rearing Events in the Elevated Plus-Maze Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the number of rearing events in elevated plus-maze following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.19, P2=0.17). Rearing event is defined as both front paws lifted up above the floor. S/E= Social and Enriched Group (n-6), S/NE= Social and

Non Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

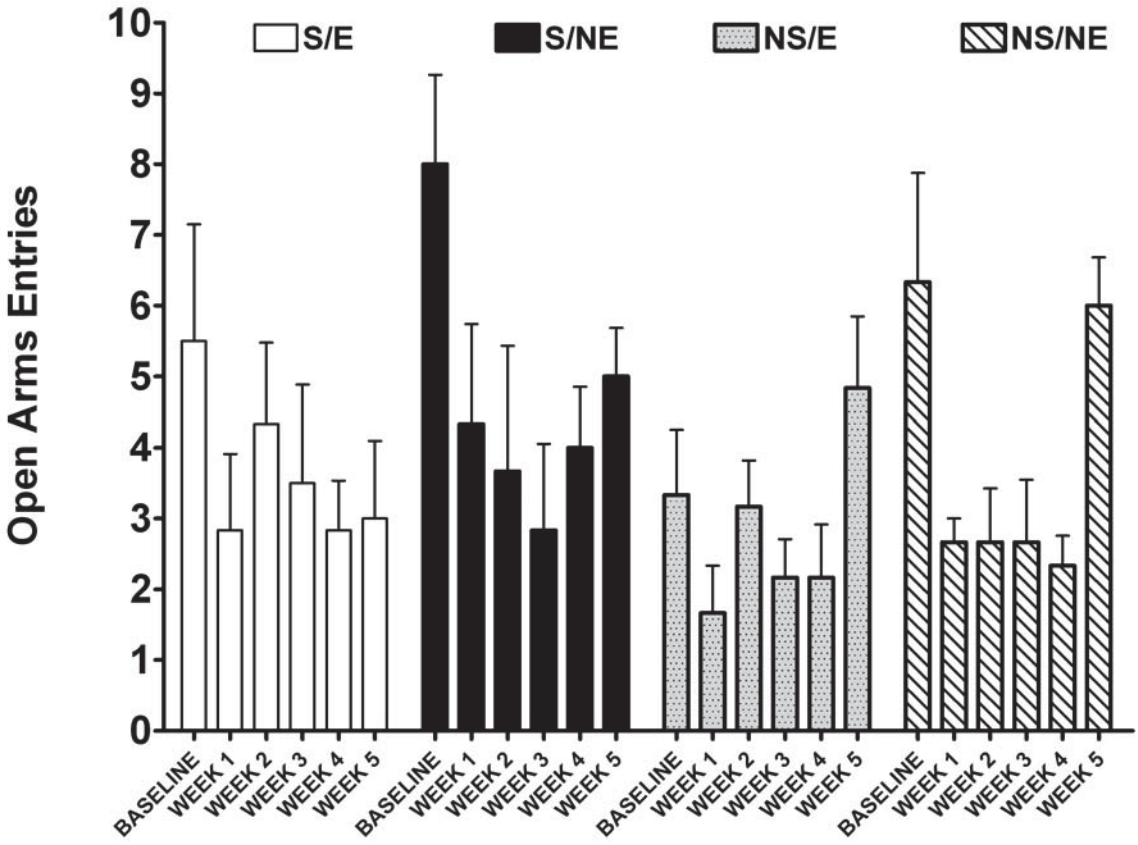

Figure 12: The Open Arms Entries in the Elevated Plus-Maze Test

Comparison of the subjects placed under four different housing conditions (S/E, S/NE, NS/E, NS/NE). The changes in anxiety-like behavior measured by the open arms entries following repeated measurement for baseline, week 1, week 2, week 3, week 4, week 5 (P1= 0.33,

P2=0.25). The open arms entries is defined as when more than 70% of the rat's body entering into open arm. S/E= Social and Enriched Group (n=6), S/NE= Social and Non

Enriched Group (n=6), NS/E=Non Social and Enriched Group (n=6), NS/NE= Non Social and Non Enriched Group (n=6).

CHAPTER 4

IV. Discussion

The modern strategy for pain treatment has been developed over the course time, due to advances in pain research. Now, a multifactoral approach to treatment has been widely accepted in patients' pain care. The scientific evidence indicates that it is not adequate to only look at the sensory component of pain; the affective and cognitive components must also be considered. As Katz (1998) pointed out, pain relief is not the only need of patients who suffer from lower back pain attacks. Psychological reassurances are equally important. Anxiety is proposed to be an important factor associated with the affective component of pain (Mok, 2008). Pain and anxiety disorders all reveal associations with social interaction and environmental variables (Katja 2010, Bao 2003, Jacobi 2004). Chronic pain has a negative affective component and is closely related to anxiety (Blier, 2001). Treating anxiety may, in turn, reduce the experience of pain (Adrienne, 2008). Edwards (2007) reported that clinic patients with high levels of anxiety suffered more symptoms of pain. Pain-related anxiety has been well documented in the clinic, but is not well understood.

In this study, we looked at how environmental cage complexity and social interaction affected anxiety level. As being understood, anxiety is linked to the affective pain that is supported by the clinical studies, and evidence of affective pain component and anxiety share the same anatomical structures known as the limbic system (Tatiana, 2004). We believe that the reduction of anxiety would lead to relief of affective pain. The results from this study indicate that there was no statistically significant correlation between environmental cage complexity and anxiety. The results of previous studies on

animal model research were varied in terms of the anxiogenic effect caused by cage complexity. Cage complexity has been shown to have a positive effect on attenuating anxiety level (Hughes, 2010). In contrast, other studies reported the opposite result, that there were no statistically significant effects on anxiety level as a function of differing cage complexity (Elliot, 2005). Thus, attempting to determine if cage complexity has a significant influence on anxiogenic effects has been controversial. As each lab used different strain that made it impossible to compare the results. And also It would be possible that cage enrichment is not the critical factor to alter the anxiety level. In order to confirm how the cage complexity affects the anxiety level, more researches need to be conducted in a consistent manner in terms of the strain used and procedures.

The important finding in the current study was that the male rats under isolation for a week demonstrated increased anxieogenic profile supported by the results from both open field test and elevated plus-maze test. In the open field test, anxiety-like behavior variables, such as the time spent in the center squares, the number of occurrences of exploring a novel object, and the number of occurrences of rearing events, all consistently demonstrated a noticeable decrease at the first week of behavioral data, after living in isolated housing conditions. The decrease in the time spent in the center square and the decrease in locomotive activities in open field all indicated higher anxiety levels. In the elevated plus-maze test, the data were consistent with the open field test results. The time spent in the open arms, as an index of anxiety level, also decreased. Interestingly, the data from open field test and elevated plus-maze test, they all support the trend that the rats housed in an isolated condition for a week demonstrated higher anxiety level than the ones in social groups. Social isolation has been understood to be a

big stress factor affecting the animals' anxiety-like behavior (Hall, 1998). Social isolation has been proposed as a model for anxiety (Parker, 1986). This current finding concurs with results of previous studies. It seems isolation housing condition promotes the higher anxiety level that is linked to the affective pain. Wilkinson (1994) claimed that being raised in isolation produced long-term behavioral alterations that were characterized by increased expression of anxiety-like behavior. Rodgers (1993) also reported results consistent with our data in terms of social housing condition attenuating the anxiety level. Prior research also has demonstrated a link between anxiety and chronic pain and utilized social factor to reduce the anxiety level. Magni (1990) found a positive correlation between anxiety and musculoskeletal pain. Gallagher (1999) found a moderate correlation between anxiety and arthritic pain. Nicholson (2004) proposed the vicious cycle of anxiety, muscle tension and pain. Our studies discovered the trend that there is an inverse relationship between social interaction of anxiogenic effect. Therefore, due to the link between pain and anxiety, we speculate that if we decrease the level of anxiety by altering social interaction, the affective pain would be attenuated. The results of the current study on social condition, supported by prior research, could inform research dealing with chronic pain.

The underlying mechanisms involved in the effects of social interaction on anxiety-like behaviors remain to be determined. The concept of social isolation functioning as "stress" was introduced by Hatch in the early 1960s (Hatch, 1963). Abnormal reactive behaviors of isolated rats were described for the first time. In his studies, socially isolated rats demonstrated very reactive behavior in response to human handling. The rats appeared nervous, aggressive and hyper-emotional (Hatch, 1963). The

early life isolation from social counterparts has been reported to constitute a stressful experience and as such, has consequences on adult anxious behaviors and hormonal reactions (Holson, 1991). Based on previous studies, the results of link between social isolation and behavior studies, and hormonal response from Hatch (1963) and Holson (1991) indicated that the social isolation variable was one of the most employed experimental stress models, which affects the hypothalamic-pituitary-adrenocortical axis (HPA) due to the increased basal level of adronocoticotropic hormone (ACTH) and Corticosterone (CORT) in plasma (Weiss, 2004). It has been proven that abnormalities in the behavioral response, including the anxiety-like behavior of isolated rats are associated with functional changes in the endocrine response (Serra, 2005). There are abundant evidences indicate early life social isolation affecting brain development and subsequent changes in endocrine system, resulting in alternation of anxiety level. The association between social isolation and anxiogenic effect is potent. A few of hypotheses can be proposed to explain the outcome of this study. We speculate that the aggregated anxieogenic profile induced by the social isolation housing condition may be partly attributed to the hyperactivity of the HPA axis. This is known to be a major endocrine system responsible for the adaptive response to stress. The HPA axis is a negative feedback system that involves interaction among the hypothalamic, pituitary, and adrenal glands. The hypothalamus links the nervous system to the endocrine system via the pituitary gland. The HPA negative feedback loop acts as checks-balances system. Stress triggers the production and secretion of corticotropic-releasing factor (CRF). Like a chain reaction, CRF stimulates the pituitary gland to secrete ATCH. The production of ACTH leads to the release of cortisol, referred to as “stress hormone”. Cortisol initiates a

negative feedback of the HPA axis system by sending a signal to the hypothalamus and the pituitary gland to inhibit the production of CRF. That decreases ACTH production, finally leading to stable levels of cortisol in the blood. It is possible that when the rats were housed in an isolated condition, it caused the excessive production of ACTH and cotisol and impaired the negative feedback regulation of the HPA axis system, which would alter the behavioral response. It is possible that as higher ACTH and cortisol in the plasma reach the certain level that the HPA axis is not able to function as negative feedback system, and impair its ability to decrease the ACTH and cortisol production. The dysfunction of the HPA axis system is hypothesized. This hypothesis is supported by the studies done by (Weiss, 2004 & Serra, 2005). The evidence from their studies showed that increased anxiety in the rats housed in isolated conditions were accompanied by altered hormonal response, such as higher basal levels of ACTH and Corticosterone (CORT).

Further, we propose the second hypothesis based on the studies demonstrated that stress decreased the number of 5HT1 receptors in the hippocampus (Popva and Petkov, 1990). The hippocampus is known as one of the important component of the limbic system that is responsible for anxiety. It is possible that dysfunction of the limbic system caused by the decreased the number of 5HT1 receptors in the hippocampus, resulting in behavioral alteration, specifically increasing anxiety level. In agreement, Guimaraes (1993) reported that social isolation reduced the 5HT1 receptors in the hippocampus, which led to limbic system dysfunction, and eventually leading to alternation of anxiety. This might provide evidence to support one of the explanations, suggesting an increased anxiogenic effect resulting from the isolation housing condition.

In the current study, all behavioral data in results of week 2 through week 5 showed the habituation effect. Habituation is a decrease in response to a stimulus after repeated exposure to the same stimulus. It is a gradual adaptation process to the environment that was demonstrated in our study by no consistent changes in anxiety-like behavior when the subjects were repeatedly placed in the open field apparatus and the elevated plus-maze from week 2 to week 5.

There were limitations in this study, which need to be addressed in future research. The sample size was small, which may be the cause of the relatively high variability in the data. Due to this limitation, sample size may potentially account for why we only see the clear trend instead of statistically significant differences in the results. Also, future research may need to conduct the open field test and the elevated plus maze test in a randomized fashion. This may possibly minimize random chance statistically. In the future we may increase the sample size. In addition, we may employ the reverse design to confirm the results. For example, the subjects would be housed in group first, and later, when the design is reversed, they would be housed individually. Further, fine manipulation of the parameter can be employed by extending the duration of the isolation condition, which may amplify the effect of social factors in influencing the anxiety level.

In summary, The present study sought to determine if environmental cage complexity and social interaction affected anxiety-like behavior. Although there were no significant differences between the cage complexity housing conditions and anxiety-like behavior, there was a noticeable trend between the nonsocial housing condition and anxiety-like behavior. The data consistently showed that the isolation condition promoted anxiety-like behavior in both the open field test and the elevated plus-maze

test. The finding of the present study may provide additional evidence for understanding the relationship between social isolation and anxiety-like behaviors. Realizing that social factors are very important in the regulation of anxiety disorders suggest that we may decrease affective pain by enhancing social interaction. This study suggests that we might need to look into the correlation between anxiety-like behavior and the hormonal response of endocrine system in the future study.

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Friday, January 29, 2010

Dr. Jill Gifford
Biology Department
UNIVERSITY

Re: IACUC Protocol # 04-09
Title: The effect of housing condition on anxiety behavior

Dear Dr. Gifford:

The Institutional Animal Care and Use Committee of Youngstown State University has reviewed the aforementioned protocol you submitted for consideration titled "**The effects of housing condition on anxiety behaviors**" and determined it should be unconditionally approved for the period of **September 28, 2009** through its expiration date of **September 28, 2012**.

This protocol is approved for a period of three years; however, it must be updated yearly via the submission of an Animal Review-Request to Use Animals form. These Annual Review forms must be submitted to the IACUC at least thirty days ***prior*** to the protocol's yearly anniversary dates of September 28, 2010 and September 28, 2011. You must adhere to the procedures described in your approved request; any modification of your project must first be authorized by the Institutional Animal Care and Use Committee.

Sincerely,

Dr. Peter J. Kasvinsky
Associate Provost for Research
Dean School of Graduate Studies and Research

PJK:dka

C: Dr. Walter Horne, Consulting Veterinarian, NEOUCOM
Dr. Robert Leipheimer, Chair IACUC, Chair Biological Sciences
Dawn Amolsch, Animal Tech., Biological Sciences