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Impact of Corticosterone and Zinc Deprivation on Memory and Hippocampal Functioning

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IMPACT OF CORTICOSTERONE AND ZINC DEPRIVATION ON MEMORY AND HIPPOCAMPAL FUNCTIONING

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This dissertation, submitted by Patricia L. Moulton in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

(Chairperson)

This dissertation meets the standards for appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

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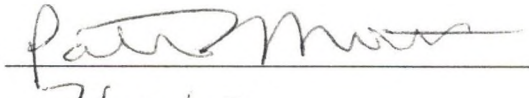
Signature 

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ABSTRACT

The purpose of this study was to examine the impact of long-term exposure to corticosterone (CC) and chronic zinc-deprivation (ZnD) on memory performance and hippocampal damage in rats. Previous work has examined the impact of exposure to CC or the impact of ZnD, but no previous work has examined the combined impact on memory performance. Previous work suggests that chronic exposure to CC or ZnD results in hippocampal damage and memory deficits.

The present study utilized a broader range of memory tests than previous studies and administered the memory tests early and late in the time period of CC exposure and ZnD. CC is released as a part of the stress response and is initially beneficial to the organism in maintaining homeostasis. However, with chronic exposure to stress and subsequent chronic CC release, damage to the cell bodies and neurons of the hippocampus have been found. This is problematic because the hippocampus plays a role in memory and learning mechanisms. Memory problems are also present in stress-related disorders such as post-traumatic stress disorder (Bremner, 1999) and depression (Sheline et al., 1996).

The hypotheses of this study were that hippocampal degeneration as a result of long-term CC exposure and ZnD would result in decreased long- and short-term spatial memory in Sprague-Dawley rats with a greater decrease in memory in ZnD rats as compared to ZnA (Zinc-adequate) animals. Degeneration of the neurons of the hippocampus would also be apparent in the CC treated animals with more degeneration

exhibited in the ZnD rats than the ZnA animals, and this would correspond to greater deficits found in memory.

Results indicated a general decrease in performance was found for the CC animals as compared to control animals on the memory tasks. A decrease in the hippocampus area CA3 pyramidal cell layer width was observed in the animals treated with CC. Performance on the radial arm maze was significantly correlated with the width of the CA3 pyramidal cell layer. Performance on the water maze was significantly correlated with the concentration of zinc in the vesicles in the CA1 and CA3 areas of the hippocampus.

CHAPTER 1

INTRODUCTION

Glucocorticoids (GCs) are a class of adrenal cortical hormones that are activated during stress. In humans the primary glucocorticoid is cortisol, in rats it is corticosterone. In later portions of the stress response, glucocorticoids are released in order to mobilize energy, increase cardiovascular functioning, to inhibit unnecessary processes (such as digestion) and attempt to regain homeostasis. The hypothalamic-pituitary-adrenal axis regulates the production of glucocorticoids. Normally, glucocorticoids are released during specific times of the circadian rhythm with increased secretion in the morning for humans and in the evening for rats, which is related to the diurnal cycle. Additional release of GCs during stress depends on the intensity, duration and type of stimulus. In individuals with tumors, symptoms of stress, depression and alcoholism increased release of GCs have also been found (Buckingham, Cowell, Gillies, Herbinson & Steel, 1997).

Carroll et al. (1981) administered a dexamethasone suppression test to 438 subjects along with a diagnostic interview to detect the presence of endogenous depression. In those subjects with symptoms indicative of depression, abnormal cortisol concentrations were found in 98% of the subjects. Young et al. (1991) found that this abnormality in cortisol levels may be due to inhibition of the glucocorticoid feedback system a system which is responsible for the regulation of the amount of circulating cortisol, this inhibition results

in abnormal levels of cortisol. In animals, stress results in reduced activity, freezing and increased startle responses (Levine & Ursin, 1991).

Corticosterone and the Hippocampus

The hippocampal formation is a portion of the limbic system and is composed of the subiculm, hippocampus and the dentate gyrus. The hippocampus is actually composed of three cell layers, the outer molecular layer contains the afferent axons and dendrites that radiate from the middle layer, the pyramidal layer contains the efferent neurons, and the inner polymorphic layer which contains the axons of the pyramidal cell neurons (Haines, 1997). The afferent (pathway between sense organ and brain) perforant hippocampal pathway exits the sensory organs and then connects to the primary and secondary sensory cortices, to the entorhinal cortex and then to the hippocampus. The fibers running from the superficial layers of the entorhinal cortex are the main afferent pathways to the hippocampus with fibers going to all three major hippocampal structures, the dentate gyrus, CA1 and CA3. The cells in the dentate gyrus in turn send mossy fibers to CA3. The CA3 then sends the Schaffer-commissural fibers to the CA1. Efferent (pathway between brain and muscles or glands) fiber pathways lead from the hippocampus, through the deep layers of the entorhinal cortex and to the ventral striatum. These two areas then send efferent fibers to the hindbrain, to the motor system and also to the dorsomedial nucleus of the thalamus, which in turn connects to the frontal cortex (Gluck & Granger, 1993).

The hippocampal formation is thought to consolidate immediate and short-term memories into long-term memories. Consolidation is thought to occur by the hippocampus using information from the sensory and motor cortices and combining them

with the memory that is being formed thus allowing us to remember the context of a particular memory (Carlson, 2001). One way this may occur is through long-term potentiation in which a synapse fires in a particular pattern in response to a particular stimuli. This pattern of firing increases the probability that the stimuli will continue to activate the targeted cell. Long-term potentiation is thought to result from the integration of several synaptic inputs and this leads to the formation of a memory (Haines, 1997).

The implication that damage to the hippocampus effects memory was first discovered in the patient H.M. who had both the left and right hippocampi surgically removed in order to control a seizure disorder. H.M. developed anterograde amnesia in which he was unable to learn and form new long-term memories (Scoville & Milner, 1957). Past research has also demonstrated that in rats, lesions of the hippocampal formation produced deficits in performance on a radial-arm maze (Handelmann & Olton; 1981 Kesner, 1985) and a 16-hole memory board task (Oades, 1981). On the radial-arm maze task used in this study the animal was assessed on whether it remembered which of several arms of a radial maze were already visited. In the 16-hole memory board task, animals are required to search for four pellets hidden in 16 holes in the testing cage. On both tasks, errors occur when the animal visits an arm or a hole that had already been visited, testing the animal's memory and use of the hippocampus. These tasks illustrate the role of the hippocampus in memory.

Effect of Corticosterone on the Hippocampus and Memory

Stress has been theorized as a cause of hippocampal damage resulting from GC release. A few days of stress endangers the hippocampus, over a few weeks, increased levels of GC secretion cause dendritic atrophy and after a few months of chronic

exposure to stress, permanent loss of hippocampal neurons occurs (Sapolsky, 1996). This loss of hippocampal neurons was demonstrated in a study by Sapolsky, Krey and McEwen (1985) who exposed Fisher 344 rats to daily injections of corticosterone in order to produce high plasma levels for three months. Extensive depletion of GC receptors, a loss of CA3 field cells, and an increase in the number of darkly stained cells in the CA3 field indicated several signs of neuronal destruction.

Several mechanisms of action have been proposed for the degeneration of hippocampal cells by GCs, including that GCs disrupt the availability of energy to hippocampal neurons by disrupting the transport of up to 15-30% of the glucose to hippocampal tissue. This percentage is very high considering that hippocampal neurons are very vulnerable, rely on glucose to survive, and cannot store glucose. GCs may also disrupt conversion of glutamate to glutamine. Glutamate is an excitatory neurotransmitter that activates the NMDA receptor, which allows excess calcium to enter hippocampal cells. Excess calcium may also lead to neuron death (Sapolsky, 1991).

Previous investigations of the direct impact of stress on hippocampal damage have also shown an indirect impact on memory. Bodnoff et al. (1995) investigated the effects of chronic corticosterone treatment on spatial memory and neuropathology of young (5 months) and mid-aged (12 months) Long-Evans rats in three experiments. In Experiment 1 corticosterone or cholesterol pellets that were implanted for one or three months and produced elevated corticosterone levels to 30 +/- 2 μg per day. In Experiment 2, rats were subjected to either social stress or a combination of stress and corticosterone implantation with 100 mg GC pellets for six months. In Experiment 3, animals were implanted with corticosterone pellets produced by Innovative Research that released a

steady dosage for three months. The rats for the Experiment 3 were divided into a medium-concentration group with average corticosterone concentrations between 12-17 μg/day, a high-concentration group with concentrations of 23-32 μg/day and a control group receiving cholesterol pellets. The rats were tested using the Morris (1984) water maze which requires the animal to find a submerged platform in a pool of opaque water using only distal cues. The dependent variables in the Morris water maze were distance swam, latency to the submerged platform and time swimming around the submerged platform. For Experiment 1, the administration of corticosterone had no effect on performance on the water maze after one month of treatment for either age groups. After three months of treatment there were significant increases for latency and distance between corticosterone-treated and cholesterol-treated mid-aged animals on the water maze, while no differences were found in young animals due to effects of corticosterone treatment, implicating an increased vulnerability with age. In Experiment 2, the group that received social stress showed higher plasma corticosterone concentrations, which were about two times the amount of control animals. The social stress animals also demonstrated impaired performance on the water maze with increased latency and swim distance. In Experiment 3, the rats that received high concentrations of corticosterone spent less time in the area around the submerged platform, impairing performance on the water maze with increased latency and distance swam. There were no significant differences found in neuron density in the CA1 and CA3 regions or cell size in Experiment 3. The medium and high-concentration corticosterone animals showed impaired excitability and short-term plasticity when tested with electrophysiological techniques. This study only examined the hippocampal tissue of the

rats in Experiment 3. The lack of neuronal loss in the hippocampus, even in the presence of behavioral deficit, may depend on the strain of rats used. The results of this study are in conflict with other studies that have found hippocampal damage when given chronic doses of GC (Sapolsky, 1991, Sapolsky, Krey & McEwen, 1985) with Fisher 344 rats. There might be differences in calcium-dependent energy stores or glutamate activity between strains that have not been identified.

Quervin, Roozendaal, and McGaugh (1998) examined the effects of stress and GCs on retrieval from long-term memory in a series of studies. In these studies, young Sprague-Dawley rats (10-12 weeks old) were trained within eight trials to locate a submerged platform in a Morris water maze (Morris, 1984). Twenty-four hours later the rats received a retention trial. Prior to the retention trial, a total of three, 8mA foot shocks with duration of one s with five-s intervals between shocks were administered. The rats received the three shocks 2-, 30- or 240-min before the retention trial. The control group did not receive any shocks. The control group spent significantly more time swimming in the quadrant of the water maze in which the platform had originally been placed than the shocked rats and exhibited focused searching patterns. The only group of rats that showed significant impairment of memory was the group that received a shock 30 min before their retention trials who demonstrated random swimming patterns. However, when the total length of swimming paths was calculated, there was no significant difference across the groups of rats. This study found increased levels of corticosterone in the 30-min group after the retention trial that was significantly higher than the other groups. A follow-up study used subcutaneous doses of 50 mg/kg of metyrapone, a drug which decreases the synthesis of corticosterone. Metyrapone was injected 40 min before

foot shock using the same Morris maze and retention trials as the previous study. The rats injected with metryapone did not exhibit significant increases in plasma corticosterone concentrations following foot shock and also did not show differences in maze performance as compared to control rats. A third study was conducted to further the understanding of GCs on memory retrieval in rats that were not exposed to stress. The same Morris water maze task was used with retention tested 24 hours later. The rats were given 0.3, 1.0 or 3.0 mg/kg of corticosterone, subcutaneously, 30 min before the retention trial. A dose-dependent increase in plasma corticosterone concentrations was found and treatment with the two higher doses (1.0 and 3.0 mg/kg) significantly impaired retention on the water maze similar to the impairment that was seen after the foot shock given at 30 min. These results demonstrated that increased concentrations of GCs might inhibit retention from long-term memory.

McLay, Freeman and Zadina (1998) administered 150 mg GC pellets (Innovative Research) for 80 days followed by testing in the Barnes (1979) circular platform maze and the Morris (1984) water maze. The Barnes circular platform maze consists of a white, .95-m diameter circle with 12 holes spaced around the edges. Under one of these holes is a darkened escape chamber, which is always in the same place although the platform itself is rotated between trials. The dependent variables of the Barnes maze included distance traveled, errors, the amount of time moving or resting and time to escape. The corticosterone-treated animals demonstrated significantly more errors, distance traveled, and time to escape. On the Morris water maze, the cortisone-treated animals also exhibited longer latency to escape. The animals also lost a significant amount of body weight due to corticosterone treatment. The authors found no other

effects of the treatment on temperature or health and also found no differences on open-field motor activity when compared to control animals.

The above research demonstrates that exposure to a stressor or to GCs results in impairment in Morris water maze and the Barnes circular platform maze performance. In addition, impaired excitability and short-term plasticity was found in medium to high corticosterone treated animals. Thus chronic exposure to stress can result in memory problems and hippocampal damage.

Effects of Zinc Deficiency on Behavior and Brain Morphology

Another variable that can be attributed to memory problems is ZnD (Zinc-deprivation), which can also lead to hippocampal damage. The hippocampal formation contains the largest concentration of Zn (Zinc) with 30-40% more Zn than other regions of the brain. In particular the CA3 area of the hippocampal formation contains the most Zn per weight. Zn concentration in the mossy fibers is 100 times higher than in cerebrospinal fluid and 20 times higher than in blood plasma. The mossy fibers form a communication link within the hippocampal formation (Crawford & Harris, 1984).

Zn, as an essential trace element, is acquired through consumption of whole grains, meat, fish and shellfish. Reduced calorie or protein intake may lead to ZnD (Golub at al., 1995). Another common cause of ZnD is the consumption of the many processed foods that are widely available today. These foods include canned or frozen vegetables in which the processing strips their Zn content, white bread, sugary foods and fatty snacks. These foods may replace Zn-rich foods. Although, the RDA (recommended daily allowance) of Zn for adult males is 15 mg/day and adult females is 12 mg/day, few Americans actually consume this amount. Penland (2000) stated "Research on beneficial

effects of improved Zn nutrition for behavior and brain function is critically needed because Zn deficiency continues to be a modern health concern in both developing and developed countries" (p. 363S).

It has been shown that ZnD during gestation, lactation and juvenile development has an impact on the hippocampus and on activity and learning (e.g. Golub at al., 1983, Halas at al., 1983). In a review of the available research on zinc deficiency, Sandstead et al. (2000), concluded that ZnD has been shown to cause malformations during early brain development. In later development, abnormalities in brain function have also been found. Kawamoto and Halas (1984) exposed female, Long-Evans rats to a ZnA diet (25 μg Zn/g), a ZnD (0 μg Zn/g) diet or pair fed (fed same amount of ZnA diet as ZnD group consumed daily) for 100 days. After testing, the rats were euthanized, perfused, and the hippocampal formation was examined. The hippocampal formation upon light and electron microscope examination appeared normal in all treatment groups. The mean area of each of eight measured sections of the hippocampal region was reduced in both the pair-fed and the ZnD rats as compared to control rats. Testing in a 17-arm maze resulted in the ZnD rats making significantly more errors than either of the other two groups. The ZnD rats also used less variety in patterns or strategies in maze running than the other two groups. A positive correlation between number of errors on the maze and the neuronal density of the dentate granule cells of the hippocampus was also found with greater neuron density associated with more errors on the maze.

One study has been done which compared the effects of ZnD between juvenile and adult rats. Gordon (1984) compared two age groups of rats, 35- and 300-day old rats on several behavioral tests to examine the effects of ZnD. Three dietary groups included a

ZnD group that received 1 ppm of Zn, a pair-fed group received 50 ppm of Zn, and a ZnA group received food containing 50 ppm of Zn. All groups were fed their prescribed diets for 52 days. A circular open-field test measured latency to leave a center circle, grooming, freezing, sectors entered, rearing and number of boli during the testing session. Both ZnD young and old groups exhibited decreased food intake, decreased body weight and decreased plasma Zn concentrations. The young ZnD rats also had conjunctivitis, alopecia, inflamed paws, urethitis and skin lesions. The older ZnD rats had only alopecia and skin lesions. The older ZnD rats had a longer latency to leave the center circle, less grooming time and entered fewer sectors of the field than the younger ZnD rats. No prior studies have examined the impact of ZnD on adult rats (> 5 months age).

Rationale for Present Study

The studies reviewed demonstrate that a stress-induced increase in corticosterone, can impact hippocampal neurons and pathways (Sapolsky, 1991). The hippocampus is an important structure in encoding and processing of time-dependent associations and spatial memories (Zola-Morgan & Squire, 1993). Research has also shown that the administration of GCs can lead to deficits in spatial memory and hippocampal damage (Bodnoff et al., 1995, Quervin, Roozendaal & McGaugh, 1998, McLay, Freeman & Zadina, 1998). However, there are limitations to these studies.

The first limitation is that most of studies only measured differences in behavior once, after administration of a stressor or GCs. In addition, many of the studies also measured the effects of an acute stressor, while the long-term effects of a chronic exposure to stress have not been examined. McLay, Freeman and Zadina (1998) did administer GC for 80 days, but then tested once, after treatment had been completed. This eliminates any

possible examination of effects at different times during treatment. Bodnoff et al. (1995) administered GC for 90 days and tested at one and three months of treatment; however, they only tested using the Morris water maze.

The present study examined the effect of corticosterone early and late in a 90-day period of treatment in order to assess short-term spatial memory (radial arm maze) and long-term spatial memory (water maze). Also, the present study incorporated the Kant (1993) water maze, a measurement of long-term memory, which has been shown to be sensitive to pharmacological interventions including amphetamine sulfate, diazepam, caffeine, and atropine sulfate.

The second limitation of previous work is that activity was not assessed in the corticosterone studies. High plasma levels of GC have been found in patients with chronic depression (Sheline et al., 1996) and in patients with post-traumatic stress disorder (Bremner, 1999). In addition, high levels of GC have been associated with impaired performance on tests of memory in humans (Keenan et al., 1996). Animal models of depression have demonstrated decreased motor activity in an open-field test (Pare, 1994). Also, ZnD rats have exhibited impaired performance on open-field activity (Gordon, 1984). Therefore, activity level should be sensitive to manipulations of both GC and Zn. In the present study, 24-hour home cage activity and open-field activity using an apparatus which evaluates movements in 3-D was used to assess activity early and late in the treatment period.

The third limitation of previous work lies in the association of brain damage produced by GC and/or Zn with behavioral deficits. Only one study has been done (Bodnoff et al., 1995) which examined behavior and direct effects of chronic CC treatment on

hippocampal tissue. Hippocampal damage was not observed. However, several other studies have found damage to the hippocampus after exposure to GC (Sapolsky, 1991; Sapolsky, Krey & McEwen, 1985) but they did not measure behavior. In addition, Kawamoto and Halas (1984) found a correlation between ZnD and hippocampal damage. The present study examined two memory and two activity tests and associated performance to damage to hippocampal tissue due to GC and Zn.

The importance and uniqueness of the present study lies in the compounding of nutritional deficiency with environmental stress. Due to our fast-paced, technology based society with increasing stressful demands; important vitamins and minerals are consumed at unacceptably low levels. Instead, many modern diets consist of highly processed foods and decreased amounts of fruit [illegible] and whole grains. One of the symptoms of depression and post-traumatic stress disorder is decreased appetite, which also may lead to decreased consumption of Zn. Zn may compound existing problems with stress and excess GC, resulting in greater amounts of hippocampal damage and indirect effects on memory.

The hypotheses of this study were that hippocampal degeneration as a result of long-term GC exposure and ZnD would (1) result in decreased long- and short-term spatial memory in Sprague-Dawley rats with a greater decrease in memory in ZnD rats as compared to ZnA animals, and (2) degeneration of the dendrites and cell bodies of the hippocampus would be apparent in the CC treated animals with more degeneration exhibited in the ZnD rats than the ZnA animals and this would correspond to greater deficits found in memory (see Figure 1).

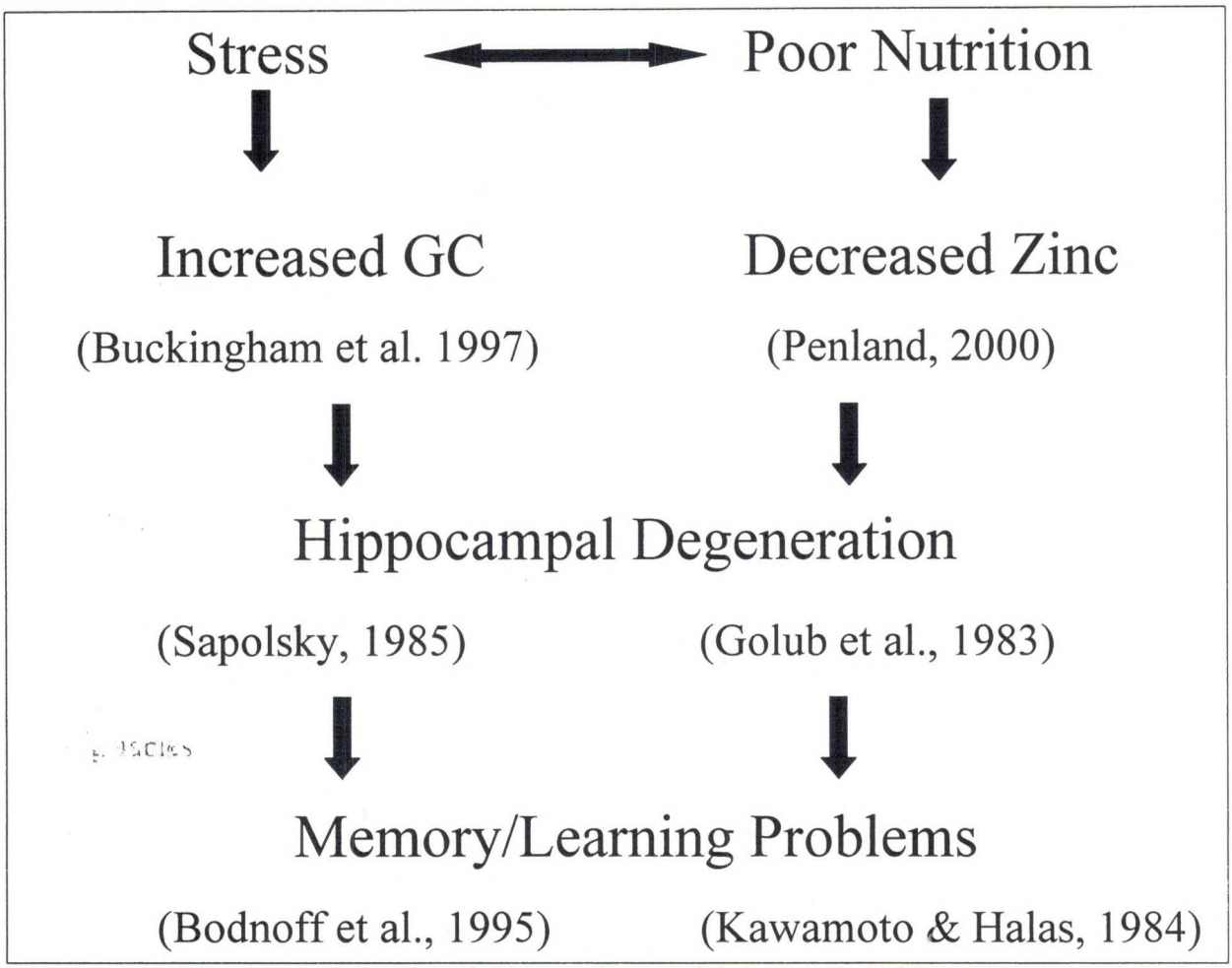

Figure 1. Diagrammatic model of the hypotheses of the present study.

CHAPTER 2

METHOD

Animals

Seventy-two male weanling Sprague-Dawley rats were ordered from SASCO (Wilmington, MA) and housed at the U.S.D.A. Human Nutrition Center (Grand Forks, ND) for five months before implementation of experimental treatments so that effects of the treatments could be examined in mature (6-month old) rats. During that first 5 months, all animals were fed a control growth diet (AIN-93G; Reeves, Nielsen & Fahey, 1993) containing 12 ppm Zn. At 6 months of age, the animals were randomly assigned to one of three experimental diets described below, all based on the AIN-93M diet (Reeves, Nielsen & Fahey, 1993), and remained on those diets for the duration of the study (13 weeks). Both diets (AIN-93G and AIN-93M) were formulated to exceed nutrient requirements published by the National Research Council (1978) for laboratory rats. De-mineralized water was available ad libitum. The rats were housed in individual metal cages under a 12-hour reversed light/dark cycle (0800-2000). The animal room was maintained at 22° C (+/- 2° C) and 45% (+/- 10%) relative humidity. The animals were matched for weight across treatment groups prior to the start of treatment. The NIH Guide for Care and Use of Animals was followed and the Animal Care and Use Committee approved the animal care protocol at the USDA-ARS Grand Forks Human Nutrition Research Center.

Materials and Apparatus

All rats were assigned to one of three dietary groups, 24 rats were fed the AIN-93M diet containing an adequate concentration of 12 ppm Zn (ZnA), 24 rats were fed the AIN-93M diet containing low concentration 1 ppm Zn (ZnD), and 24 rats were fed the ZnA diet containing 12 ppm Zn but intakes were restricted to intakes of matched rats in the ZnD group (pair-fed; PF). Because of the known problem with weight loss associated with ZnD (Gordon, 1984), the pair-fed group provided a control group for food consumption. The rats were fasted by removing their food the evening prior to the radial arm and Tru-Scan activity testing. Again, de-mineralized water was available ad libitum. The food pellets used as motivation/reward in the radial arm maze and the Tru-Scan activity Monitor consisted of sucrose (Bioserve, Frenchtown, NJ).

The CC treatment groups received sustained corticosterone exposure through 2 subcutaneous implanted 200 mg-corticosterone pellets in the upper back (total dose 400 mg) (Innovative Research, Sarasota, Florida), designed to release corticosterone at 4.44 mg/day for 90 days. This dose was the same as used by Bodnoff et al. (1995). The control groups received a sham surgery. Any minor rise in CC levels as a result of the surgery was expected to diminish during the one-week recovery period and should not have affected behavioral testing or had permanent effects on hippocampal tissue. Plasma CC levels were determined monthly from 3 ml of blood taken from the tail and using a HPLC procedure described below. Behavioral testing utilized Coulbourn Instrument's modular testing equipment. The 22-hour Home Cage Monitoring System included individual infrared sensors placed on each rat's home cage. The sensors continually record small and large movements and the duration of movement for 22-hour blocks of

time. The Tru-Scan Activity Monitoring Chamber consists of three sensor rings capable of tracking movement in X, Y, and Z planes. The chamber also has a 16-hole nose poke floor. The eight-arm radial maze (Olton & Samuelson, 1976) has three-feet long runways attached to a central hub controlled with guillotine doors that are computer controlled. Each entryway contains a photo-beam sensor as well as a pellet feeder trough at the end of each runway. The Kant (1993) water maze consists of a large water tank (5'diameter), painted white with non-toxic paint. White, opaque plastic walls, 50 cm high form a series of concentric squares with doorways in the center of the walls. Alleys between the walls are 16 cm wide. A movable, square platform extends above the water line, to provide an escape platform. The maze was filled to the height of 25 cm with 24 ° C water and this temperature was maintained throughout the study. A white curtain surrounds the maze and cue shapes are placed around the curtain. The maze was video taped and the tapes were analyzed for latency and number of errors. Boli were also collected in order to monitor the stress status of each rat. All behavioral testing equipment was wiped clean with a sanitizing solution between animals in order to reduce the possibility of odor cues.

Procedure and Sequence

Near the end of the 5-month growth period, the rats were handled once weekly by the experimenter in order to habituate the rats to handling. Experimental diets were introduced at 6-months during the week of CC pellet implantation. De-mineralized water was freely available to the rats in their home cage. According to Sapolsky (1995) and Mclay et al. (1998), when elevated CC concentrations are administered for one week or more, possible side effects include weight loss, opportunistic infections, and loss of calcium in the bones. ZnD has also been found to cause anorexia in rats. Due to these

concerns, food consumption and weight loss were monitored. The animals were weighed every Wednesday during the study by vivarium staff. Daily food consumption was assessed by vivarium staff on a daily basis and pair feeding was done until it was determined that pair-feeding was unnecessary due to little difference in weight or food consumption between the dietary groups. Pair-feeding was ended on the same day for both groups, resulting in the end of pair feeding at two months for group A and at 6 weeks for group B. Food consumption was done 5 days a week at this time until the end of the study. Both CC exposure and ZnD may also increase the opportunity for illness due to decreased immune function. Every attempt was made to reduce the possibility of illness through cooperation with veterinarian and vivarium staff and the use of universal precautions. The schedule for the study is included in Appendix A. All behavioral tasks, surgical procedures, blood draws, necropsy and perfusion procedures were practiced using five scrub animals prior to experimentation.

Behavioral Task Training

During the first two weeks of the study, the animals received training on the radial-arm maze and the water maze. For the radial-arm maze, in the first two days of training, sucrose pellets were scattered throughout the eight arms (three pellets per arm) and the fasted rats (fasted since 10:00 P.M. the night prior to training) were allowed 15 min to explore the arms. For the last three days, the ends of all eight arms were baited once, and the fasted rats were allowed to freely move in the maze for 15 min (Handelemann & Olton, 1981). Water maze training occurred during week two. Each animal was allowed to swim from the center starting point in the maze until it reached the finish platform placed in the outer rim of the maze or five min had elapsed. Those animals that did not

reach the platform in five min were pushed gently with a paddle through the maze to the finish platform. All animals received three trials each day with 30 s between trials. The animals were trained for five consecutive days or until they reached criterion. Those animals that successfully reached the platform in two consecutive trials within 100 s were considered trained. Animals were counterbalanced across training conditions in that half of the animals from each group received radial-arm maze training and then water maze training during week two. The other half of the animals received the reverse order of training.

Corticosterone Administration and Monitoring

After training, corticosterone pellets were implanted in six rats per day for a total of twelve days. The animals were assigned implantation numbers matched across groups with animals 1-6 implanted on day one, animals 7-12 on day two, through animals 67 - 72 on day twelve. The animals were anesthetized with an isoflurane (4%) and oxygen mixture by using an ANESCO anesthesia machine and Ohio vaporizer (chamber induced and nose cone maintained). Once the animal was anesthetized, the animal's upper back between the shoulder blades was shaved with an electric trimmer. The shaved area was then wiped twice with Betadine to prevent infection. To implant the pellets, the shaved area was wiped with an alcohol wipe to get rid of any dirt. The animal's level of anesthesia was then checked by pinching the animal's foot and ears. If there was no reaction, surgery began. If there was a reaction, the level of anesthesia was slightly increased. The skin on the upper back of the animal was pinched to separate it from the underlying muscle and a small incision was made with dissecting scissors. A pocket on each side of the spinal cord was then made with blunt edge scissors and two pellets were

implanted on each side. The edges of the animals skin was then gathered up and stapled shut with wound clips. Any blood was cleaned up using a gauze pad. Sham animals received the same incision and the wound was stapled shut without any pellets implanted. Blood was drawn from the tail in order to measure baseline corticosterone concentrations in all animals. The animal's tail was dipped in warm water to dilate the blood vessels in the tail. The animal was then placed back on the table dorsal side up, with its nose in the anesthesia machine nose cone. The animals body was placed in a straight line and the index finger of the experimenter's left hand pressed down hard on the animal's tail to stop the flow of blood, and expose the vein. The tail vein appears as a white-pink line running down the middle of the tail. The 3-ml syringe with a 22-gauge needle was inserted at a 45-degree angle in the area of the tail vein in order to locate the tail artery, which is directly below the vein. When the tail artery is reached, blood began filling the syringe and when possible 3-ml of blood was collected. The blood was then placed in 5-ml falcon tubes containing EDTA, an anti-coagulant. The tube was closed and carefully mixed. All blood samples were centrifuged for 15 minutes at 3000 rpm, plasma separated and then kept in a -80°C Revco freezer. The animals were then kept warm after surgery and monitored until they awakened. The animals were allowed to recover from the operation for one week. Corticosterone concentrations in plasma was measured once every four weeks during the study for a total of three times, following the same order as implantation to provide consistent CC exposure for all animals.

Behavior Testing Sequence

All animals followed the same treatment and testing sequence, but, for logistical reasons they were run in two groups staggered one week apart. Animals were tested for

the first session of 22-hour home cage monitoring during week 2, session one of the radial-arm maze during week 3, session one of the water maze during week 5, the Tru-Scan Activity monitoring during week 9, the second session of the 22-hour home cage monitoring during week 10, the second session of the radial-arm maze during week 11 and the second session of water maze during week 12. All behavioral testing (with the exception of 22-hour activity testing) occurred in the same order as the implantation schedule with six animals per day in order to provide consistent CC exposure for all animals. Animals were tested between 9:00 A.M. and 1:00 P.M. (except for 22-hour activity testing) in order to control for any effects of time-of-day. During the breaks in the behavioral testing sequence the animals were left in their home cage. The tests were distributed throughout the study to more specifically examine the chronic versus acute effects of CC exposure.

The 22-Hour home cage monitoring system was set to provide continuous monitoring of activity for two complete days. Eighteen rats were monitored at one time. The entry of laboratory technicians for feeding and health monitoring into the room in which the rats were housed was indicated on a log.

Tru-Scan activity monitoring sessions were 60-min long in which the fasted rats (fasted since 10:00 P.M. night before testing) were measured for latency and errors in collecting sucrose pellets placed in the 16-hole floor during the first 30 minutes of the session. During the second half of the session, a 1500-Hz tone was sounded at 30, 40 and 50 min for 20 seconds. The computer measured many variables of activity, rearing, and nose-poke.

For the water maze testing, the animals were placed in the same starting area as the training sessions and allowed five min to find the exit platform. The animals were tested for three trials each with a 30 s rest period between each trial.

For the radial-arm maze, the fasted animals were placed in the center box of the eight-arm radial maze with all of the guillotine doors closed. After 30 s, all eight doors opened at once. After the rat had traveled to the end to the end of an arm, received the reinforcer, one sucrose pellet and the animal had returned to the starting area; all eight doors were again closed followed by a 10 s delay. Then all eight doors were again opened and the animal was allowed to choose among all eight arms, including those that had already been visited. This sequence continued until the animal had visited all eight arms and received a sucrose pellet or 15 min have elapsed. The animals received three test trials separated by one hour between each trial during which the animal was returned to its home cage.

Necropsy, Perfusion and Examination of Hippocampal Tissue

Necropsy and perfusion occurred following the same six rats per day schedule with half of the rats each day euthanized at the Grand Forks Human Nutrition Research Center (A.M.) and half of the rats perfused at the UND Anatomy and Cell Biology Department (P.M.). The animals were divided between the two locations according to the condition of the implanted pellets throughout the study. Several animals' pellets became exposed and had to be re-implanted on one or more occasions. Those animals that had no repair surgery or only minor surgeries were classified as "perfect", only one repair surgery was classified as "less than perfect" and those animals with multiple repair surgeries were classified as "bad" (see Table 1). This was done in order to maximize the potential for

animals with good corticosterone pellet implantations to be evenly distributed at both sites.

Table 1. Division of Animals between Necropsy and Perfusion

	Zinc- Adequate Diet				
	CC (Perfect)	CC(LP)	CC (Bad)	Total CC	Sham-surgery
Necropsy	2	3	6	11	13
Perfusion	5	2	5	12	10
	Zinc –Deprived Diet				
	CC (Perfect)	CC(LP)	CC (Bad)	Total CC	Sham-surgery
Necropsy	2	2	2	6	6
Perfusion	4	0	2	6	6

Note. P= perfect, defined as no repair surgeries or only 1 minor surgery
LP= less than perfect, only 1 repair surgery
B= bad, multiple repair surgeries

For the necropsy at the Nutrition Center, animals were deeply anesthetized with a euthanizing dose of Ketamine and Rhompin. Ten ml of blood was then collected through a heart puncture and placed in a 10 ml tube containing EDTA. Two ml of this blood was used for cell count analysis using a Cell-Dyne apparatus (Abbott Laboratories, Illinois). The remaining eight ml of blood was separated and 1.5 ml of the plasma was frozen for corticosterone concentration measurement. The remaining plasma was also frozen and sent to the Trace Mineral Lab at the Grand Forks Human Nutrition Lab for analysis of concentration of trace minerals. The animals were then decapitated and the brain, liver and femurs were removed, weighed and frozen for trace mineral analysis. The trace mineral analysis of the blood, brain, liver and femurs was unavailable for this paper. The

brains were cut in half through the corpus callosum and 1/2 was retained for total brain mineral concentrations. The other 1/2 was sent to Neurobiotex (Galveston, TX) for analysis of brain-zinc-vesicle concentration.

For those animals selected for perfusion, they were brought over to the Anatomy Department on the day of perfusion. The animals were deeply anesthetized with an euthanizing dose of sodium pentobarbital. The animal's chest cavity was opened and prior to perfusion, 2-3 ml of blood was collected through the right atrium from an exposed heart. This blood was placed in a 5ml tube containing EDTA. This blood was separated and 1.5 ml of the plasma was frozen and used for analysis of corticosterone concentrations. The remaining plasma was sent to the Trace Mineral Lab at the Grand Forks Human Nutrition Center for analysis of trace mineral concentrations. The animals were then injected with a prefix solution containing 1000 µl/ml heparin and 1% sodium nitrite with a 22-gauge needle placed in the left ventricle of the heart. The animals were perfused for about 10 minutes at a rate of 10-15 mls/min with 4% paraformaldehyde with a total of 200 ml of fixative perfused per animal. The brains were then removed and placed in 4% formaldehyde for one hour as a secondary fixative. They were then transferred to a 15% sucrose/formalin solution for cryoprotection and placed in a refrigerator.

Histological examination of the hippocampus was done at the University of North Dakota Anatomy and Cell Biology Department. Twelve animals were selected for analysis with three animals per dietary group (zinc-adequate and zinc-deprived) and three animals per treatment group (corticosterone implant and sham surgery). The corticosterone implanted animals were those that were classified as either "perfect" or

"less than perfect" in order to reduce any noise from multiple implant surgeries. These twelve brains were each sliced in half with the left half of the brain used for analysis. The right half of the brain was returned to the refrigerator to be used at a later date. The left half of the brain was placed on a sliding, freezing microtome and 40 μm slices were made through the entire thickness of the brain. These slices were placed in a potassium buffered solution (PBS) (80% double-distilled water :20% phosphate buffer). Fifteen slices from each animal was then mounted on gelatinized slides and allowed to dry. These slices were then rinsed in the PBS solution, stained with a toulidine blue stain for 30 seconds and then the excess staining solution was rinsed off with PBS. After the slides dried, they were coverslipped with a 1:1 glycerol solution.

Examination of the zinc-concentrati[illegible]t Neurobiotex (Galveston, Texas) using the 6-methoxy 8-p-toluene sulfonamide quinoline (TSQ) fluorescence method for staining zinc in brain zinc vesicles (Frederickson, Kasarskis, Ringo & Frederickson, 1987).

Determination of Plasma Corticosterone Concentrations

The measurement of plasma concentrations of corticosterone was done following a high-performance liquid chromatography procedure as outlined in a paper by Dawson, Kontur and Mojan (1984). Standards for the HPLC including corticosterone, 11-deoxycorticosterone, hydrocorticosterone (cortisol), cortisone and cortisone acetate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo). Standards were made up in mobile phase as stock solutions (250 μg/ml) and stored at 0°C. The mobile phase consisted of 77.5% 18 meg ohm (ultra-pure) water, 12% tetrahydrofuran and 10.5% acetonitrile. The mobile phase was flask filtered using a 45 μm Nylaflo nylon membrane

filter. This mobile phase was delivered using a Shimadzu LC-10AD pump module and was set at a flow rate of 2 ml/min during detection. Separation occurred on a 4.5 X 250 mm Supelcosil LC-8 5 analytical column which was protected by a 4.6 X 50 mm LC-8 Pelliguard column. Both columns were purchased from the Supelco division of Sigma-Aldrich (St. Louis, MO). Column temperature was maintained at 50°C by a Shimadzu CTO-10AC column oven. Samples were auto injected using a Shimadzu SIL-10A auto-injector. Detection occurred using a Shimadzu variable wavelength UV-VIS detector set a 239 nm. All HPLC modules were controlled using a Shimadzu SCL-10A system controller. Data was integrated and recorded using a Shimadzu CR-501 Chromatopac. Peak height and internal standardization was used for quantification. Standard calibration curves were [illegible].

All blood samples were centrifuged for 15 min at 3000 rpm, plasma separated and then kept in a –80°C Revco freezer. On the day each sample was processed it was removed from the freezer, kept on ice and thawed for 1 hr. Approximately 20 samples were processed each day. First, each sample was vortexed for 5 s in order to thoroughly mix the plasma. One ml of plasma was then pipetted into a 3-ml glass tube and 2-ml of the internal standard consisting of 100% methanol containing 100 ng of dexamethasone was added to each tube. This mixture was then vortexed for 30 s which served to deproteinate the plasma and extract protein-bound and free steroids. The mixture was then placed in a refrigerated centrifuge for 5 min at 3000 rpm and low brake. After spinning down, the supernatant that has been separated out was poured into another 3-ml glass tube, which contained 2-ml of 18 meg ohm water and vortexed for 5 s to mix thoroughly. This solution was poured into separate 3-ml C-18 extraction columns using a

vacuum manifold. The samples were then washed with 2 washes of 1.0-ml volume of 18 meg ohm water, followed by 1 wash of 1.0-ml of 10% methanol solution, and then 2 washes of 1.0-ml of 20% acetone solution. The samples were then eluted into 1.5-ml epi tubes with two washes of .5 ml 100% methanol and then dehydrated in a Supervac for approximately 3 hours or until thoroughly dry. During this period the C-18 columns were regenerated for the next day's use by doing 3 washes of 3-ml each of 100% methanol. When the samples were dry they were then reconstituted with 100 µl of 100% methanol and syringe filtered into 100 µl HPLC vials with screw tops and seals and placed into the HPLC apparatus to be auto-injected into the column. Standards were injected and analyzed first each day followed by one 20 µl injection of each sample.

CHAPTER 3

RESULTS

This study incorporated a 3 (diet) X 2 (treatment) X 2 (time) mixed factor design with one within-subjects factor and two between-subjects factors. The between-subjects factors were zinc and corticosterone while the within factor was time. The dependent variables for this design were 22-hour activity monitoring (two sessions), water-maze testing (two sessions), radial-arm maze (two sessions), Tru-Scan activity monitoring, serum corticosterone levels (baseline and three testings), cell count of the hippocampal cells and width of pyramidal cell layer of the CA1 and CA3 areas of the hippocampus, the calculation of zinc concentration in vesicles in the hilus, CA1, CA3 areas of the hippocampus, the cortex and thalamus, total weight and food consumption, whole blood cell count and trace mineral analysis of plasma, brain, liver and femurs. Analysis was run separately for each variable and are presented below, starting with the physiological measurements, followed by the behavioral measures and then an overall correlational analysis comparing blood and brain values with results from behavioral testing. In instances of the violation of the assumption of sphericity for ANOVAs, the Greenhouse-Geisser corrected ANOVA was used and reported. In all occasions when a significant effect was found regardless of correction method, the Greenhouse-Geisser was not used and is not reported in this paper.

Corticosterone Concentrations

HPLC samples were matched with standards using retention times. Retention time is the amount of time the sample is exposed to the UV wavelength before a peak is detected. Those samples which did not match standard retention times were discarded. Concentrations were determined by running a linear regression on three levels of each standard for each day of the experiment and the constant and x coefficient derived from this regression used to compute concentrations (μM) using the following formula: Plasma concentration = (area of peak of sample – constant) / x coefficient.

Table 2. Mean Corticosterone Concentration: Time X Treatment

	Treatment = Corticosterone Implant	
	Dietary Condition	
	Zinc-adequate	Zinc-deprived
Baseline	17.29 (6.66)	18.56 (2.64)
One Month	13.21 (6.27)	14.47 (3.10)
Two Months	13.43 (5.93)	14.18 (2.20)
Three Months	16.95 (10.75)	13.76 (3.95)
	Treatment = Sham Surgery	
	Dietary Condition	
	Zinc-adequate	Zinc-deprived
Baseline	16.47 (3.22)	17.12 (3.83)
One Month	14.68 (3.05)	15.79 (1.90)
Two Months	14.41 (3.69)	16.25 (2.59)
Three Months	20.86 (3.51)	18.97 (4.33)

Note. Parentheses indicate standard deviation. Concentrations are in μM.

The only steroid which demonstrated significant differences was corticosterone. The other steroid measurements resulted in few determined concentrations, resulting in a very low sample size making analysis difficult on these measurements. The other steroids are not presented here. Means and standard deviations for corticosterone can be found in table 2.

Table 3. Source Table for Corticosterone Concentration

Source	SS	df	MS	F	Prob.	Power	Eta^2
Time	133.83	3	44.61	11.37	.00*	1.00	.45
Time X Treatment	62.20	3	20.73	5.28	.00*	.91	.27
Time X Diet	39.32	3	13.11	3.34	.03*	.72	.19
Time X Treatment X Diet	2.53	3	.85	.22	.89	.09	.02
Error	164.81	42	3.92				

Note. * = $p < .05$.

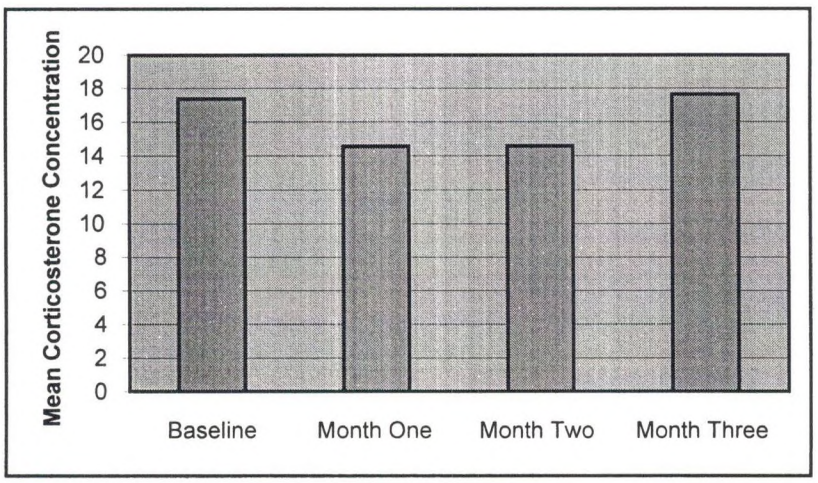

Figure 2. Time of Corticosterone Draw.
Measurements were taken at baseline, one month, two months and at necropsy/perfusion at three months.

A 2 (Treatment) X 2 (Diet) X 4 (Time) mixed ANOVA resulted in a main within-subjects effect of Time with $F(3, 42) = 11.37$, $p < .01$ (see Table 3). Higher mean values

were found at Baseline (M=17.36 μM) and Three months (M=17.64 μM) than at Month 1 (M= 14.54 μM) and Month 2 (M=14.57 μM) draws (see Figure 2).

A significant within-subjects interaction of Time X Treatment, F (3,42) = 5.28, p = .003 was also found (see Figure 3). A subsequent analysis using t-tests (2-tailed) revealed that for the corticosterone-implant group the highest concentration was found at baseline (M = 17.92 μM) with this draw significantly higher than all three of the other draws (one month M = 13.84 μM; two months M = 13.81 μM; and three months M = 15.36 μM). For the sham surgery group the highest concentration was found at the three-month draw (M = 19.91 μM) with this draw significantly higher concentration than all three of the other draws (baseline M = 16.79 μM ; one month M = 15.23 μM; and two months M = 15.33 μM).

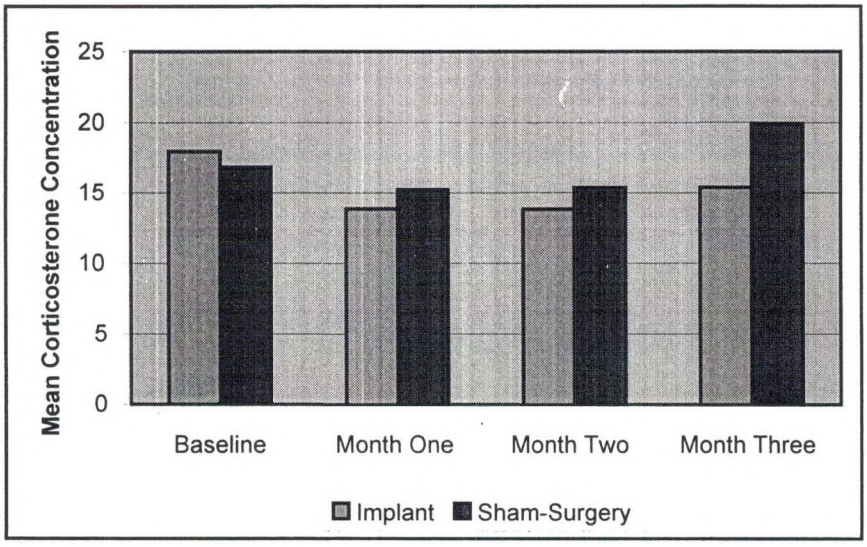

Figure 3. Time X Treatment for Corticosterone Concentrations. Measurements were taken at baseline, one month, two months and at necropsy/perfusion at three months.

A significant within-subjects interaction of Time X Diet was found, F (3,42) = 3.34 p= .028 (see figure 4). A subsequent analysis using t-tests (2-tailed) of this interaction

revealed that for the zinc-adequate animals the highest corticosterone concentration was at 3 months (M = 18.90 μM) and a there was a significant difference between this draw and all three of the other measurements (baseline M = 16.88 μM; one month M = 13.94 μM and two months M = 13.92 μM). For the Zinc-deprived animals the highest concentration was found at baseline (M = 17.84 μM) with this draw significantly different from the one month (M = 15.13 μM) and two month draws (M = 15.22 μM) and no significant difference between month three and the other draws. The zinc-adequate animals also had a significantly higher concentration at the three month draw (M = 18.90 μM) than the Zinc-deprived animals (M = 16.37 μM) with no other differences between diet groups for the baseline, one month and two month draws.

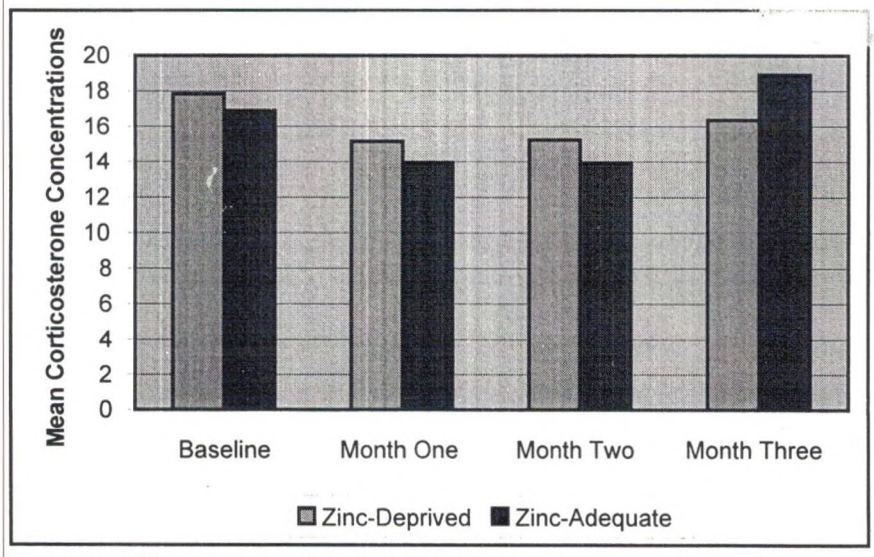

Figure 4. Time X Diet for Corticosterone Concentrations. Measurements were taken at baseline, one month, two months and at necropsy/perfusion at three months.

In order to determine if the location of the last blood draw played a factor in this analysis an independent samples t-test was used to compare the three month blood draw for the animals which had been perfused at the Anatomy and Cell Biology Laboratory

with those animals which underwent necropsy at the Nutrition Center. This test was important because half of the animals were sampled in the morning during the necropsy at the Nutrition Center and the other half were sampled in the afternoon during the perfusions at the Anatomy and Cell Biology department. This difference in sample times could potentially lead to confounding effects of time of day as corticosterone levels fluctuate throughout the 24-hour cycle with the lowest level found in the afternoon on a reversed light-dark cycle. Another potentially confounding effect may be differences in environmental setting as levels of stress hormones are extremely susceptible to environmental stressors. The T-test was not significant, t (51) = -1.391, p = .170 (2-tailed). In addition, those animals which had received more than one reimplantation surgery during the study were compared with animals which did not require implantation. A t-test resulted in no significant differences between these two groups for all 4 measurements of corticosterone, baseline: t (10) = .58, p = .58 (2-tailed), one month: t (24)= -.86, p = .40 (2-tailed), two months: t (25) = -.518, p = .61 (2-tailed) and three months: t (26) = -1.77, p = .09 (2-tailed). Thus multiple re-implantations did not have a significant effect on corticosterone concentration.

Weekly Weights and Food Consumption

Average weight for each animal for the entire treatment period and average food consumed was determined and used for group analysis. A 2 (Treatment; Corticosterone-implant, Sham-surgery) X 3 (Diet; Zinc-adequate, Zinc-deprived, Pair-fed) ANOVA resulted in significant differences for average weight for treatment group, F (1,65) = 12.94, p = .001 and for average food consumption for treatment group, F (1,65)= 5.28, p = .03. The corticosterone implant group was significantly heavier (M= 537.86g) and

consumed more food (M= 21.98g) than the sham surgery group (weight M=508.71g; food M= 21.22g). There were no significant differences for dietary group or for the interaction of treatment and dietary group.

Table 4. Mean Weight: Treatment X Diet

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	537.38 (40.47)	511.45 (29.83)
Zinc-deprived	539.80 (35.34)	503.23 (28.77)

Note. Parentheses indicate standard deviation. Weights are listed in grams.

Table 5. Source Table for Mean Weight

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	15517.34	1	15517.34	13.11	.00*	.95	.16
Diet	133.88	1	133.88	.11	.74	.06	.00
Treatment X Diet	449.43	1	449.43	.38	.54	.09	.01
Error	79333.74	67	1184.09				

Note. *= $p < .01$.

With the pair-fed animals collapsed into the zinc-adequate groups, a 2(Treatment) X 2 (Diet) ANOVA also resulted in a significant differences for the Treatment group for average weight, $F(1,67) = 13.11$, $p = .001$ and for food consumption $F(1,67) = 7.27$, $p = .009$. In this analysis the corticosterone implant group weighed more (M= 538.59g) and consumed more food (M= 22.13g) than the sham surgery group (weight M = 507.34g; food M = 21.20g). There were no significant differences for dietary group or the interaction between dietary group and treatment. Means and standard deviations for this

second analysis are presented in Tables 4 and 5 (weight) and tables 6 and 7 (food consumption).

Table 6. Mean Daily Food Consumption: Treatment X Diet

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	21.69 (1.34)	21.28 (1.16)
Zinc-deprived	22.57 (1.88)	21.11 (1.28)

Note. Parentheses indicate standard deviation. Consumption is listed in grams.

Table 7. Source Table for Daily Food Consumption

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	13.84	1	13.84	7.27	.01	.76	.10
Diet	2.06	1	2.06	1.08	.30	.18	.02
Treatment X Diet	4.37	1	4.37	2.30	.13	.32	.03
Error	127.60	67	1.91				

Note. *= $p < .01$.

Necropsy Organ Weights and Hematology

A 2(Treatment) X 2(Diet) ANOVA on the necropsy body and organ weights resulted in several significant differences for treatment. The total body weight for the corticosterone-implant animals ($M = 555.46$g) was significantly greater than the sham surgery animals ($M = 524.07$g), $F(1, 32) = 4.77$, $p = .04$. Liver weight was also significantly greater in the corticosterone treated animals ($M = 16.08$g) than sham-

surgery animals (M = 14.16g), F (1, 32) = 6.57, p = .02. (see Tables 8 and 9) No significant effects of diet or interactions between treatment and diet were found.

Table 8. Mean Necropsy Animal and Organ Weights: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-Adequate	Zinc-deprived	Zinc-Adequate	Zinc-deprived
Body Weight	569.84 (40.64)	541.08 (43.93)	535.35 (37.52)	512.79 (48.85)
Brain Weight	2.38 (.09)	2.33 (.17)	2.28 (.09)	2.27 (.12)
Liver Weight	16.05 (2.43)	16.12 (2.34)	14.91 (2.34)	13.41 (1.16)

Note. Parentheses indicate standard deviation. Weights are listed in grams.

Table 9. Source Table for Necropsy Animal and Organ Weights

Source		SS	df	MS	F	Prob.	Power	Eta^2
Treatment	Weight	8428.46	1	8428.46	4.77	.04*	.56	.13
	Brain	.05	1	.05	4.09	.05	.50	.11
	Liver	31.80	1	31.80	6.57	.02*	.70	.17
Diet	Weight	5632.54	1	5632.54	3.19	.08	.41	.09
	Brain	.01	1	.01	.62	.44	.12	.02
	Liver	4.36	1	4.36	.90	.35	.15	.03
Treatment X Diet	Weight	82.14	1	82.14	.05	.83	.06	.00
	Brain	.01	1	.01	.48	.50	.10	.02
	Liver	5.21	1	5.21	1.08	.31	.17	.03
Error	Weight	56494.95	32	1765.47				
	Brain	.41	32	.01				
	Liver	154.92	32	4.84				

Note. * = $p < .05$.

Complete blood count was assessed to ascertain the health of the animals at the end of the study. Sapolsky (1995) and McLay et al. (1998) found side effects of corticosterone administration including opportunistic infections. In addition, zinc-deprivation has been

associated with depressed immune system function. Complete blood cell count includes measurements of white blood cells which can indicate whether the immune system has been activated in the presence of virus, bacteria or allergens. The complete blood count (CBC) was measured by a Cell-Dyne apparatus (Abbot Laboratories, Illinois) and measurements included red blood cell count, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count and mean platelet volume (MPV). Normal values listed in the tables are from Veterinary Hematology (Schalm & Jain, 1986).

Table 10. Mean Whole Blood Cell Counts: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery		Normal
	Zinc-Adequate	Zinc-deprived	Zinc-Adequate	Zinc-deprived	
RBC	7.99 (.43)	7.55 (.24)	7.98 (.41)	7.07 (.52)	8.20 (.56)
Hemoglobin	13.61 (.97)	13.88 (.22)	13.38 (.80)	12.40 (1.40)	15.00 (.90)
Hematocrit	41.61 (2.56)	41.34 (1.20)	41.26 (2.60)	37.30 (3.94)	Not Available
MCV	52.03 (1.89)	54.82 (.95)	51.62 (3.08)	52.63 (1.77)	57.60 (3.40)
MCH	17.06 (1.00)	18.40 (.49)	16.78 (.47)	17.50 (.69)	18.30 (1.1)
MCHC	32.80 (1.24)	33.58 (.58)	32.34 (1.39)	33.23 (.21)	31.90 (1.20)
RDW	15.40 (1.11)	14.78 (.62)	15.10 (.79)	15.47 (1.42)	Not Available
Platelet	840.29 (227.66)	798.80 (146.99)	699.60 (203.54)	886.67 (294.25)	1,108.00 (193.00)
MPV	5.98 (.76)	6.18 (.36)	6.73 (.33)	5.91 (.30)	Not Available

Note. Parentheses indicate standard deviation.

An abnormal concentration of red blood cells and hemoglobin may be indicative of an impairment in the oxygen and waste carrying capacity of the blood or an animal with anemia (see table 10). Red blood cell count (RBC), a measure of the number of red blood cells in the sample, was significantly greater in zinc-adequate animals ($\underline{M}$ = 7.99) than the Zinc-deprived animals ($\underline{M}$ = 7.31), $\underline{F}$ (1,16) = 12.87, $\underline{p}$ = .00.

Table 11. Source Table for Whole Blood Cell Counts

Source		SS	df	MS	F	Prob.	Power	Eta^2
Treatment	RBC	.27	1	.27	1.69	.21	.23	.10
	Hemoglobin	3.35	1	3.35	4.38	.05	.50	.22
	Hematocrit	22.04	1	22.04	3.42	.08	.41	.18
	MCV	7.69	1	7.69	1.78	.20	.24	.10
	MCH	1.58	1	1.58	2.89	.11	.36	.15
	MCHC	.74	1	.74	.65	.43	.12	.04
	RDW	.17	1	.17	.18	.68	.07	.01
	Platelet	3184.07	1	3184.07	.07	.80	.06	.00
	MPV	.27	1	.27	.94	.35	.15	.06
Diet	RBC	2.08	1	2.08	12.87	.00*	.92	.45
	Hemoglobin	.58	1	.58	.76	.40	.13	.05
	Hematocrit	20.46	1	20.46	3.18	.09	.39	.17
	MCV	16.52	1	16.52	3.82	.07	.45	.19
	MCH	4.86	1	4.86	8.89	.01*	.80	.36
	MCHC	3.20	1	3.20	2.78	.12	.35	.15
	RDW	.07	1	.07	.08	.79	.06	.01
	Platelet	24188.59	1	24188.59	.53	.48	.11	.03
	MPV	.44	1	.44	1.52	.24	.21	.09
Treatment X Diet	RBC	.24	1	.24	1.49	.24	.21	.09
	Hemoglobin	1.77	1	1.77	2.31	.15	.30	.13
	Hematocrit	15.50	1	15.04	2.41	.14	.31	.13
	MCV	3.61	1	3.61	.83	.38	.14	.05
	MCH	.44	1	.44	.81	.38	.14	.05
	MCHC	.01	1	.01	.01	.91	.05	.00
	RDW	1.11	1	1.11	1.15	.30	.17	.07
	Platelet	59617.39	1	59617.39	1.30	.27	.19	.08
	MPV	1.18	1	1.18	4.12	.06	.48	.21
Error	RBC	2.58	16	.16				
	Hemoglobin	12.25	16	.77				
	Hematocrit	103.07	16	6.44				
	MCV	69.20	16	4.33				
	MCH	8.75	16	.55				
	MCHC	18.39	16	1.15				
	RDW	15.46	16	.97				
	Platelet	736292.10	16	46018.26				
	MPV	4.60	16	.29				

Note. * $p < .05$.

Mean cell hemoglobin (MCH), an measurement of the average concentration of hemoglobin in the sample of red blood cells, was significantly greater in the Zinc-deprived animals (M = 17.95) than in the zinc-adequate animals (M = 16.92), F (1, 16) = 8.89, p = .01. (see table 11).

To compare the whole blood cell counts with the normal values, the mean normal values plus/minus the standard deviation was compared against the counts obtained in this study. For red blood cell count, the implant, Zinc-deprived animals (M = 7.55) was at least one SD below normal and the sham-surgery, Zinc-deprived animals (M = 7.07) was at least two SD below normal. For hemoglobin, the implant, zinc-adequate animals (M = 13.61) and the implant, Zinc-deprived animals (M = 13.88) along with the sham-surgery, zinc-adequate animals were at least one SD below normal. The sham-surgery, Zinc-deprived animals (M = 12.40) were two SD below normal. For MCV the implant, zinc-adequate (M = 52.03), sham-surgery, zinc-adequate (M = 51.62) and the sham-surgery, Zinc-deprived (M = 52.63) animals were at least one SD below the mean.

For MCH, the implant, zinc-adequate (M = 17.06) animals were at least one SD below normal whereas the sham-surgery, zinc-adequate (M =16.78) animals were at least two SD below the normal mean. For MCHC the implant, zinc-adequate (M = 32.80) , implant, Zinc-deprived (M = 33.58) and the sham-surgery, Zinc-deprived animals (M = 33.23 were at least one SD above the normal mean.

For platelet count the implant, zinc-adequate (M = 840.29), the implant, Zinc-deprived (M = 798.80) and the sham-surgery, Zinc-deprived (M = 886.67) were at least one SD below the normal mean. The sham-surgery, zinc-adequate (M = 699.60) were at least two SD below the normal mean.

The Cell-Dyne machine measured not only total white blood cell count, but also the percentage of several types of white blood cells; neutrophils, lymphocytes, monocytes, eosinophils and basophils. Normal values are from Veterinary Hematology (Schalm & Jain, 1986) (see table 12).

Table 12. Mean White Blood Cell Counts: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery		Normal
	Zinc-Adequate	Zinc-deprived	Zinc-Adequate	Zinc-deprived	
White Blood Cell Count	3.62 (1.69)	4.79 (1.49)	5.66 (2.28)	2.99 (2.01)	Not Available
Neutrophil %	33.86 (8.84)	29.66 (10.76)	11.39 (2.43)	23.65 (10.87)	25.36 (7.50)
Lymphocyte %	52.11 (12.28)	58.34 (16.33)	80.43 (3.20)	56.48 (11.22)	70.60 (8.0)
Monocyte %	11.86 (6.48)	10.23 (6.31)	7.05 (2.51)	16.33 (9.63)	2.30 (1 8[illegible])
Eosinophil %	1.26 (.49)	1.06 (.60)	.63 (.51)	2.06 (1.45)	1.60 (1.2)
Basophil %	1.01 (1.11)	.70 (.80)	.49 (.20)	1.53 (1.10)	.10 (.31)

Note. Parentheses indicate standard deviation

Several significant effects for treatment were found. For total white blood cell count a significant Treatment X Diet interaction was found, $F(1, 18) = 5.82$, $p = .03$. Subsequent analysis revealed that the sham-surgery, zinc-adequate animals ($M = 5.66$) had significantly more white blood cells than the sham-surgery, Zinc-deprived animals ($M = 2.99$), and the corticosterone-implant, zinc-adequate animals ($M = 3.62$) with no difference for the corticosterone-implant, Zinc-deprived animals.

For the percentage of lymphocytes, there was a significant effect of treatment $F (1, 18) = 5.50$, $p = .03$ with a significantly greater percentage in the sham surgery animals ($M = 68.45$) than in the corticosterone implanted animals ($M = 55.23$). A significant Treatment X Diet interaction was also found, $F (1, 18) = 7.16$, $p = .015$. For the sham-surgery

animals the Zinc-deprived animals (M= 56.48) had less lymphocytes than the zinc-adequate animals (M = 80.43). The sham-surgery, zinc-adequate animals (M = 80.43) also had more lymphocytes than the corticosterone-implanted, zinc-adequate (M = 52.11) and corticosterone-implant, Zinc-deprived animals (M = 58.34). Lymphocytes produce antibodies against foreign materials such as viruses and bacteria.

Table 13. Source Table for White Blood Cell Counts.

Source		SS	df	MS	F	Prob.	Power	Eta^2
Treatment	Total	.07	1	.07	.02	.89	.05	.00
	Neutrophil	1031.91	1	1031.91	12.09	.00*	.91	.40
	Lymphocyte	889.92	1	889.92	5.50	.03*	.60	.23
	Monocyte	2.13	1	2.13	.05	.83	.06	.00
	Eosinophil	.18	1	.18	.30	.59	.08	.02
	Basophil	.11	1	.11	.14	.72	.06	.01
Diet	Total	2.91	1	2.91	.90	.36	.15	.05
	Neutrophil	82.68	1	82.68	.97	.34	.15	.05
	Lymphocyte	399.70	1	399.70	2.47	.13	.32	.12
	Monocyte	74.59	1	74.59	[illegible]	[illegible]	.24	.09
	Eosinophil	1.90	1	1.90	[illegible]	[illegible]	.40	.15
	Basophil	.67	1	.67	.81	.38	.14	.04
Treatment X Diet	Total	18.76	1	18.76	5.82	.03*	.63	.24
	Neutrophil	344.82	1	344.82	4.04	.06	.48	.18
	Lymphocyte	1159.13	1	1159.13	7.16	.02*	.72	.29
	Monocyte	151.33	1	151.33	3.46	.08	.42	.16
	Eosinophil	3.41	1	3.41	5.78	.03*	.62	.24
	Basophil	2.33	1	2.33	2.81	.11	.36	.14
Error	Total	58.072	18	3.23				
	Neutrophil	1536.23	18	85.35				
	Lymphocyte	2912.90	18	161.83				
	Monocyte	788.23	18	43.79				
	Eosinophil	10.60	18	.59				
	Basophil	14.92	18	.83				

Note. *= $p < .05$.

For the measurement of neutrophil percentage, a significant effect of Treatment was found, $F(1, 18) = 12.09$, $p = .00$ with a significantly greater percentage in corticosterone-implanted animals (M = 31.76) than sham-surgery animals (M = 17.52). Neutrophils serve to help defend against bacteria.

For the percentage of eosinophils, a significant Treatment X Diet interaction was found, $F(1, 18) = 5.78$, $p = .03$. Subsequent analysis revealed that the sham-surgery,

Zinc-deprived animals (M = 2.06) had a significantly greater eosinophil percentage than the sham-surgery, zinc-adequate animals (M = .63), the corticosterone-implant, zinc-adequate animals (M = 1.26) and the corticosterone implant, Zinc-deprived animals (M= 1.06). Eosinophils contribute to the allergic response (see table 13).

To compare the white blood cell values with the normal values, the mean normal value plus/minus the standard deviation was compared against the counts obtained in this study. For neutrophil percentage, the implant, zinc-adequate (M = 33.86) animals were at least one SD above normal, whereas the sham-surgery, zinc-adequate (M = 11.39) animals were at least one SD below the normal mean.

For lymphocyte percentage the implant, Zinc-deprived (M = 58.34) and the sham-surgery, Zinc-deprived (M = [illegible]) animals were at least one SD below the normal mean. The implant, zinc adequate animals (M = 52.11) was at least two SD below the mean and the sham-surgery, zinc-adequate (M = 80.43) animals were at least one SD above the mean.

For monocyte percentage, all of the groups were at least two SD above the normal mean with the sham-surgery, Zinc-deprived animals (M = 16.33) deviating the most from the normal mean. For basophil percentage, all of the animals deviated at least one SD from the mean with the sham-surgery, Zinc-deprived animals (M = 1.53) deviating the most from the mean.

Brain Histology

Three animals from each group were randomly selected for analysis of effects of treatment on the hippocampus. For each animal, three sections were examined. The CA3 area which is just beyond the curve in the hippocampus was selected on each

section. The number of complete cells were counted within the boundaries on an intra-ocular ruler (125μm). A cell was counted if it was complete with a visible nucleus (white area), if it was attached to the pyramidal cell layer band and if the entire cell fell within the boundaries of the area being counted. Pyramidal layer width was determined by taking three measurements in the area that was counted using an intra-ocular ruler. Cells were counted and width was measured at 40x power and was done blind to condition.

Table 14. Width Measurement of CA3 Section of Hippocampus by Treatment

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	70.93 μm (6.67)	88.33 μm (10.29)
Zinc-deprived	74.63 μm (5.28)	93.15 μm (25.91)

Note. Parentheses indicate standard deviation.

All nine measurements for each rat (3 measurements for each slice X three slices per animal) for cell count and pyramidal band width were averaged and these averaged numbers were used for analysis. Means and standard deviations for pyramidal band width and cell count are in tables 14 and 15.

Table 15. Cell Count of a 125 μm Section of CA3 Hippocampus

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	26.11 μm (1.49)	26.63 μm (1.19)
Zinc-deprived	27.78 μm (2.03)	28.78 μm (2.84)

Note. Parentheses indicate standard deviations.

Table 16. Source Table for CA3 Hippocampus Width Measurement

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	968.00	1	868.00	4.56	.065	.47	.36
Diet	54.42	1	54.42	.26	.63	.07	.03
Treatment X Diet	.93	1	.93	.00	.95	.05	.00
Error	1699.07	8	212.38				

A 2 (Treatment) X 2 (Diet) between subjects ANOVA on the average width measurements resulted in no significant differences (see table 16). Due to low sample size and the potential for large variability between subjects a log10 transformation on the width measurement resulted in a significant effect of Treatment, $\underline{F}$ (1,8) = 5.46, $\underline{p}$ = .048 (see tables 17 and 18). The sham surgery animals had a significantly wider pyramidal band (log10 $\underline{M}$ = 1.952) than the corticosterone animals (log10 $\underline{M}$ = 1.86) (see Figures 2 and 3). A 2(Treatment) X 2 (Diet) ANOVA of the cell counts revealed no significant effects.

Table 17.Log 10 Transformed Width Measurement of CA3 section of Hippocampus by Treatment

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	1.85 μm (.04)	1.94 μm (.05)
Zinc-deprived	1.87 μm (.03)	1.96 μm (.11)

Note. Parentheses indicate standard deviation.

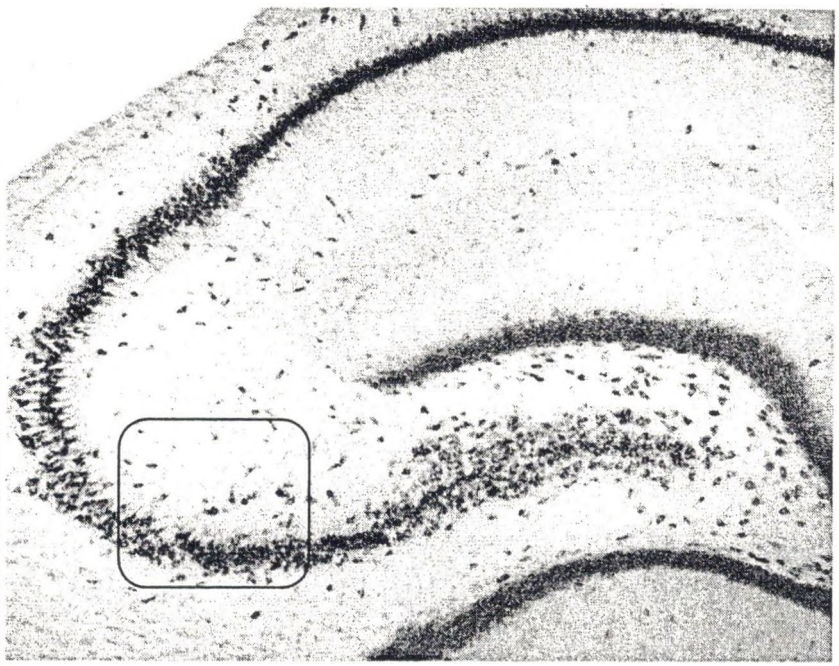

Figure 6. Photomicrograph of Hippocampus Section (40X) of an Implant/Zinc-Deprived Animal.
Circled area indicates section that was measured of CA3.

Table 19. Width Measurement of CA1 section of Hippocampus by Treatment

	Treatment Condition	
	Corticosterone-Implant	Sham-Surgery
Zinc-Adequate	47.87 µm (11.44)	55.92 µm (4.01)
Zinc-deprived	60.28 µm (26.28)	68.62 µm (13.45)

Note. Parentheses indicate standard deviation.

For the analysis of the zinc concentration of brain sections the raw data provided by LaBuda and Frederickson of Neurobiotex, Inc. included the measurement of the TSQ fluorescence intensity for 10 sections for each animal for each of the following brain areas: hippocampus hilus, hippocampus CA1, hippocampus CA3, cortex and the

Table 16. Source Table for CA3 Hippocampus Width Measurement

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	968.00	1	868.00	4.56	.065	.47	.36
Diet	54.42	1	54.42	.26	.63	.07	.03
Treatment X Diet	.93	1	.93	.00	.95	.05	.00
Error	1699.07	8	212.38				

A 2 (Treatment) X 2 (Diet) between subjects ANOVA on the average width measurements resulted in no significant differences (see table 16). Due to low sample size and the potential for large variability between subjects a log10 transformation on the width measurement resulted in a significant effect of Treatment, $\underline{F}$ (1,8) = 5.46, $\underline{p}$ = .048 (see tables 17 and 18). The sham surgery animals had a significantly wider pyramidal band (log10 $\underline{M}$ = 1.952) than the corticosterone animals (log10 $\underline{M}$ = 1.86) (see Figures 2 and 3). A 2(Treatment) X 2 (Diet) ANOVA of the cell counts revealed no significant effects.

Table 17. Log 10 Transformed Width Measurement of CA3 section of Hippocampus by Treatment

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	1.85 µm (.04)	1.94 µm (.05)
Zinc-deprived	1.87 µm (.03)	1.96 µm (.11)

Note. Parentheses indicate standard deviation.

Table 18. Source Table for Log10 Transformed Width Measurement

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	.02	1	.02	5.46	.048*	.54	.41
Diet	.00	1	.00	.23	.64	.07	.03
Treatment X Diet	.00	1	.00	.01	.92	.05	.00
Error	.04	8	.00				

Note. * =p <.05.

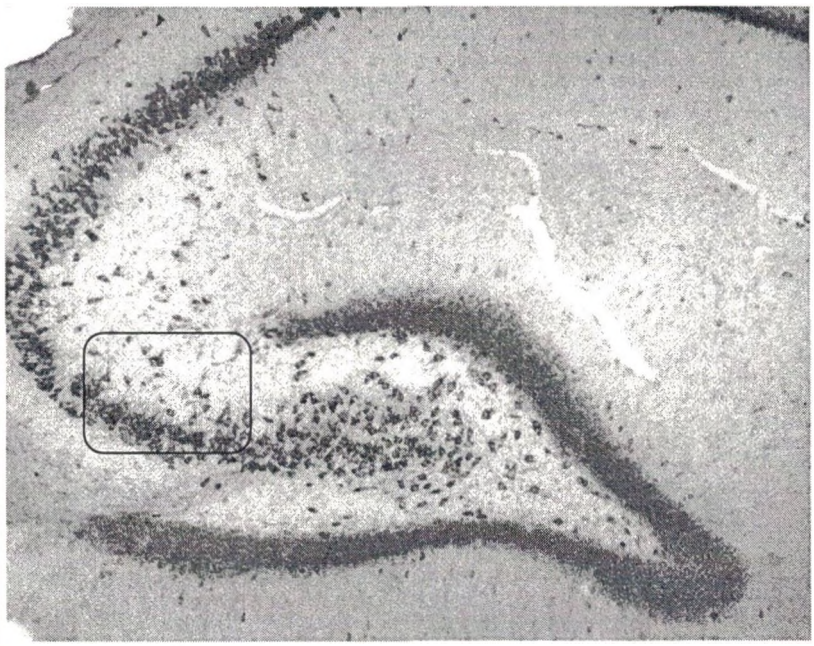

Figure 5. Photomicrograph of Hippocampus Section (40X) of a Sham-surgery/Zinc-Adequate Animal.
Circled area indicates section that was measured of CA3.

The width of the CA1 area of the hippocampus was measured using the same procedure as for the CA3 area. A 2 (Treatment) X 2 (Diet) ANOVA of the width measurements resulted in no significant differences for treatment, diet or the interaction of treatment X diet. Means and standard deviations are in table 19.

thalamus was used. Background fluorescence was also measured and then subtracted from the original intensity measurements resulting in an adjusted intensity measurement. The measurement of intensity indicates the amount of vesicular zinc staining reflecting the pool of vesicular zinc in that particular area. The 10 sections for each brain area was averaged for each animal and this number adjusted for background, was used in the analysis. Means and standard deviations are presented in table 20.

Table 20. Mean Adjusted Cortex TSQ Fluorescence Intensity by Treatment

	Treatment Condition	
	Corticosterone-Implant	Sham-Surgery
Zinc-Adequate	19.73 (7.61)	16.76 (8.64)
Zinc-deprived	20.71 (6.17)	26.83 (4.07)

Note. Parentheses indicate standard deviation.

A 2 (Treatment) X 2 (Diet) ANOVA resulted in a significant main effect of Diet for the adjusted measurement of the Cortex, F (1, 32)= 4.72, p = .04 (see table 21) with the Zinc-deprived animals exhibiting more vesicular zinc (M = 23.77) than the zinc-adequate animals (M = 18.24). No other measurements were significant.

Table 21. Source Table for Mean TSQ Fluorescence Intensity of Adjusted Cortex Measurement

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	20.53	1	20.53	.38	.54	.09	.01
Diet	252.58	1	252.58	4.72	.04*	.56	.13
Treatment X Diet	170.72	1	170.72	3.19	.08	.41	.09
Error	1712.11	32	53.50				

Note. *= $p < .05$.

A square root transformation was also done, the means and standard deviations for the cortex measurement is in table 22. A 2 (Treatment) X 2 (Diet) ANOVA resulted in no significant differences.

Table 22. Mean Adjusted Square Root Transformed Cortex TSQ Fluorescence Intensity by Treatment

	Treatment Condition	
	Corticosterone Implant	Sham Surgery
Zinc-Adequate	4.67 (.32)	3.97 (.30)
Zinc-deprived	4.50 (.36)	5.17 (.39)

Note. Parentheses indicate standard deviation.

Table 23. Source Table for Mean Adjusted Square Root Transformed Cortex TSQ Fluorescence Intensity

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	.00	1	.00	.00	.95	.05	.00
Diet	2.04	1	2.04	2.29	.14	.31	.08
Treatment X Diet	3.62	1	3.62	4.06	.05	.49	.13
Error	1712.11	32	53.50				

Note. * =p <.06.

Long-term Activity

Long-term activity was measured in the animal's home cages using Coulbourn Instruments 22-hour Home Cage Activity Monitoring System. Activity was measured for two consecutive days during each session and occurred in two sessions, during weeks 2 and 10 of treatment. Monitoring began at 9:00 A.M. and ended between 8:00 and 9:00 A.M. the next day.

Variables measured included time spent in no movement, small movement (greater than 0 seconds minimum criterion duration) and large movement (.5 seconds criterion duration). The number of incidents of no movement, small and large movement was also calculated.

Due to problems with the software, the activity program measured roughly 16 hours per session. In order to ensure total measurement of activity was consistent across animals, only the first 450 2-min intervals for each day were used in this analysis. The total activity time and incidence for each variable was calculated for each day. For each session, both days were combined resulting in an overall measurement for each of the two sessions. Means and standard deviations are shown in tables 24 (total movement) and 25 (total time).

Table 24. Mean Total Movement on Long-term Activity: Treatment X Diet

	Treatment = Corticosterone Implant			
	Dietary Condition			
	Zinc-Adequate		Zinc-deprived	
	Session 1	Session 2	Session 1	Session 2
Small Movement	6160.35 (1137.38)	5571.82 (774.51)	5970.33 (845.31)	5074.44 (795.51)
Large Movement	3419.12 (786.12)	3447.71 (746.81)	3558.44 (790.23)	3376.22 (924.58)
	Treatment = Sham Surgery			
	Zinc-Adequate		Zinc-deprived	
	Session 1	Session 2	Session 1	Session 2
Small Movement	6180.50 (858.00)	5295.94 (691.40)	5590.78 (942.10)	5246.11 (832.05)
Large Movement	3482.56 (745.44)	3417.50 (594.65)	2990.56 (669.37)	3318.89 (992.54)

Note. Standard deviations are in parenthesis.

A 2(Treatment) X 2 (Diet) X 2 (Time) mixed ANOVA for the total small movements during a session resulted in a main effect for Time, F (1, 49) = 38.35, p = .00 with animals exhibiting more small movements in session one (M = 5975.49) than session two (M = 5297.08). A 2(Treatment) X 2(Diet) X 2 (Time) mixed ANOVA was calculated for total time spent in small movements. A significant main effect of time was found, F (1, 49) = 41.92, p = .00 with more time spent in small movements during session one (M = 2093.02) than session two (M = 1794.11).

Table 25. Mean Total Time on Long-Term Activity: Treatment X Diet

	Treatment = Corticosterone Implant			
	Dietary Condition			
	Zinc-adequate		Zinc-deprived	
	Session 1	Session 2	Session 1	Session 2
No Movement	97288.94 (2615.45)	96240.06 (2723.28)	96552.11 (2875.72)	96031.33 (3235.91)
Small Movement	2173.12 (461.67)	1897.82 (299.76)	2092.44 (338.71)	1724.78 (300.93)
Large Movement	7817.94 (2249.11)	9142.12 (2505.22)	8635.44 (2690.01)	9523.89 (2975.53)
	Treatment = Sham Surgery			
	Zinc-adequate		Zinc-deprived	
	Session 1	Session 2	Session 1	Session 2
No Movement	96516.06 (2811.61)	95731.22 (3042.20)	98527.89 (2239.60)	96340.00 3897.39)
Small Movement	2161.83 (354.23)	1770.40 (259.70)	1944.67 (377.13)	1783.44 (338.13)
Large Movement	8602.11 (2518.73)	9778.39 (2821.56)	6807.44 (1907.33)	9156.56 (3628.50)

Note. Standard deviations are in parenthesis.

A 2(Treatment) X 2(Diet) X 2 (Time) mixed ANOVA was also calculated separately for total large movements and total time spent in large movements. A significant main

effect for total time spent in large movements was found, $F(1, 49) = 18.18$, $p = .00$ with a greater amount of time spent in session two ($M = 9400.24$) than session one ($M = 7965.74$). No significant differences were found for total large movements.

A 2(Treatment) X 2(Diet) X 2 (Time) mixed ANOVA was also calculated time spent in no movement. A significant main effect of time spent in small movements was found, $F(1, 49) = 9.74$, $p = .00$ with greater time in session one ($M = 97221.25$) than session two ($M = 96085.65$).

Table 26. Mean Square Root Transformed Total Small Movement: Treatment X Diet

	Dietary Condition			
	Zinc-adequate		Zinc-deprived	
	Session 1	Session 2	Session 1	Session 2
Corticosterone-Implant	78.16 (7.38)	74.47 (5.26)	77.11 (5.31)	71.04 (5.57)
Sham-Surgery	78.43 (5.53)	72.63 (4.73)	74.54 (6.30)	72.22 (5.90)

Note. Parentheses indicate standard deviations.

A square-root transformation was done on total small movements (see table 26). A 2 (Treatment) X 2 (Diet) X 2 (Time) mixed ANOVA was again calculated and a significant main effect of time was again found, $F(1, 49) = 39.30$, $p = .000$ with animals again exhibiting more small movements during session one ($M = 77.06$) than session two ($M = 72.59$). A significant three-way interaction of Treatment X Diet X Time was also found, $F(1,49) = 4.22$, $p = .045$ (see table 27).

Subsequent t-test (2-tailed) analysis resulted in several significant differences for total small movements. The corticosterone-implanted, zinc-adequate and Zinc-deprived animals had significantly more small movements during session one (zinc-adequate

M=78.16; Zinc-deprived M = 77.11) than session two (zinc-adequate M = 74.47; Zinc-deprived M = 71.04). During session two the corticosterone- implanted zinc-adequate animals (M = 74.47) had significantly more small movements than the corticosterone-implanted Zinc-deprived animals (M = 71.04).

For the sham surgery animals, the zinc-adequate animals had significantly more small movements during session one (M = 78.43) than session two (M = 72.63). The sham-surgery, zinc-adequate animals (M = 78.43) also had more small movements during session one than the sham-surgery, Zinc-deprived animals (M = 74.55). The sham-surgery, zinc-adequate animals also had more small movements during session one (M = 78.43) than the sham-surgery, Zinc-deprived animals during session two (M = 72.22).

Table 27. Source Table for Square Root Transformed Total Small Movement

Source	SS	df	MS	F	Prob.	Power	Eta^2
Time	474.77	1	474.77	39.30	.00*	1.00	.45
Time X Treatment	3.96	1	3.96	.33	.57	.09	.01
Time X Diet	1.84	1	1.84	.15	.70	.07	.00
Time X Treatment X Diet	50.99	1	50.99	4.22	.045*	.52	.08
Error	591.93	49	12.08				

Note. *= $p < .05$.

Short-Term Activity

Short-term, open-field activity was measured using a Coulbourn Instruments Tru-Scan Monitoring System. In each one hour session, 42 variables were measured and recorded each second for each animal. In the apparatus, a grid of infra-red beams (16) covered the entire area of the cage, creating coordinates of beams with one inch between each

intersection of the beams. Every time the animal crossed a beam resulting in a beam break it was counted as a movement episode as the animal moved through another coordinate. The variables consisted of various measurements resulting from beam breaks. An example would be the movement of the animal across the floor of the apparatus. The beam breaks would take into account the movement of the animal in both the X (width) and Y (direction) resulting in a measurement of episodes of XY movement. All variables were collapsed into 5- minute intervals using means, resulting in twelve 5-min time intervals during the 60-minute session. A 1500-hertz tone was sounded for 20 seconds at the beginning of time interval 7, 9 and 11. Fifteen of the available variables were analyzed for this study including XY movement and time, XY distance traveled, Margin time, Margin distance traveled, S[illegible] movements, Stereotypic time, Vertical breaks, Vertical time, Front half entries, Front half time, Back half entries, Back half time, Clockwise-revolutions and Counter-clockwise revolutions. In addition to these variables, several measurements related to the overall session nose poke task were also analyzed, along with physiological measurements. These variables are presented individually below.

XY Movement Time

XY movement time is the cumulative duration of X-Y movement episodes. It is a measurement of the amount of time spent in horizontal movement. A 2 (Treatment) X 2 (Diet) X 12 (Time) mixed ANOVA resulted in a significant main effect of Time, $\underline{F}$ (11,693) = 69.812, $\underline{p}$ = .000 with XY movement generally decreasing throughout the session (see Table 28).

Table 28. Mean XY Movement Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	45.50 (8.27)	44.22 (6.30)	42.73 (8.06)	41.20 (11.55)
Interval 2	36.28 (10.34)	34.22 (12.57)	38.79 (7.97)	36.68 (9.87)
Interval 3	30.57 (13.35)	35.94 (7.87)	33.84 (11.08)	33.27 (10.48)
Interval 4	30.62 (10.76)	30.64 (9.87)	33.56 (11.75)	29.90 (13.73)
Interval 5	25.39 (13.74)	28.06 (11.33)	31.87 (11.72)	31.00 (10.47)
Interval 6	24.90 (12.17)	27.30 (8.85)	32.75 (9.42)	23.27 (11.35)
Interval 7	21.42 (10.62)	18.12 (10.17)	23.05 (12.38)	17.62 (10.78)
Interval 8	17.21 (10.06)	15.30 (9.96)	22.37 (13.37)	15.95 (12.22)
Interval 9	15.57 (12.54)	9.38 (6.45)	15.94 (10.50)	14.07 (11.00)
Interval 10	14.17 (9.68)	12.26 (5.74)	16.90 (11.24)	16.67 (14.70)
Interval 11	13.21 (8.64)	13.50 (9.09)	18.36 (12.65)	15.98 (10.59)
Interval 12	18.28 (9.82)	21.78 (10.48)	19.60 (10.34)	20.13 (11.71)

Note. Parentheses indicate standard deviation.

XY Movement Episodes

XY Movement episodes is the total number of episodes of XY coordinate changes without at least one sample-interval break. It is a measurement of the number of occasions of horizontal movement without the animal stopping. A 2(Treatment) X 2 (Diet) X 12(Time) mixed ANOVA resulted in a significant main effect of number of XY movement episodes, $F(11,693) = 12.578$, $p = .00$, with the number of episodes exhibiting

a drop in movement after the first sound presented in interval seven with a progressive drop the rest of the session (see Table 29).

Table 29. Mean XY Movement Episodes: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	6.98 (1.54)	7.68 (1.08)	7.69 (1.28)	6.53 (1.19)
Interval 2	8.40 (1.44)	9.00 (2.08)	8.15 (1.59)	8.88 (1.92)
Interval 3	9.00 (2.52)	10.28 (1.25)	8.94 (1.95)	9.05 (2.28)
Interval 4	8.77 (1.81)	10.10 (2.19)	9.08 (2.60)	8.55 (2.91)
Interval 5	8.23 (2.87)	8.80 (1.81)	8.75 (1.95)	8.83 (1.99)
Interval 6	8.30 (2.36)	9.60 (2.12)	9.63 (1.12)	8.33 (2.87)
Interval 7	8.08 (3.09)	7.70 (3.40)	8.44 (3.61)	6.88 (3.27)
Interval 8	7.11 (3.09)	6.64 (1.58)	8.02 (3.05)	6.57 (3.73)
Interval 9	6.17 (3.48)	4.86 (2.79)	6.71 (2.69)	6.82 (4.41)
Interval 10	6.23 (3.30)	6.46 (2.52)	7.28 (4.37)	6.20 (3.98)
Interval 11	6.14 (3.22)	6.22 (2.78)	7.21 (3.88)	6.80 (3.63)
Interval 12	8.10 (3.43)	8.38 (2.59)	8.13 (3.38)	7.50 (3.15)

Note. Parentheses indicate standard deviation.

XY Distance

XY distance is the sum of distances between successive coordinates for each movement episode. It is a measurement of how far the animal traveled. A 2 (Treatment) X 2 (Diet) X 12 (Time) mixed ANOVA resulted in a significant main effect of XY

distance, F (11, 693)= 101.81, p= .000 with distance traveled gradually decreasing throughout the session (see Table 30).

Table 30. Mean XY Distance: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	66.21 (19.61)	59.32 (12.13)	55.91 (18.19)	60.50 (25.26)
Interval 2	41.42 (16.14)	35.97 (18.14)	46.38 (17.19)	40.83 (20.34)
Interval 3	29.46 (16.11)	36.06 (14.10)	36.17 (18.72)	33.72 (16.47)
Interval 4	29.47 (14.54)	27.12 (12.39)	33.52 (17.57)	29.81 (18.76)
Interval 5	23.02 (17.58)	24.97 (14.23)	33.01 (15.97)	28.24 (14.31)
Interval 6	21.83 (14.83)	22.86 (11.88)	31.41 (14.87)	18.64 (11.51)
Interval 7	16.97 (9.93)	13.04 (8.62)	19.93 (14.05)	12.63 (8.62)
Interval 8	12.72 (9.50)	11.29 (9.72)	18.37 (13.98)	12.09 (11.80)
Interval 9	11.89 (12.03)	6.01 (4.51)	11.40 (9.70)	9.66 (8.96)
Interval 10	10.12 (8.09)	7.80 (4.30)	12.08 (9.33)	13.37 (14.35)
Interval 11	9.03 (7.01)	9.29 (8.23)	14.10 (12.29)	12.71 (12.72)
Interval 12	13.41 (10.01)	16.53 (9.83)	14.10 (9.96)	15.09 (11.19)

Note. Parentheses indicate standard deviation.

Margin Time

Margin time is the cumulative time spent within a 2-beam margin of the walls of the cage. It is a measurement of how much time the animal spent at the edge of the cage. A 2 (Treatment) X 2 (Diet) X 12(Time) mixed ANOVA resulted in a significant main effect

of time spent in the margin, F (11,693) = 9.32, p = .00, with time spent in the margin gradually increasing throughout the session (see Table 31).

Table 31. Mean Margin Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	23.92 (13.45)	20.12 (8.14)	18.29 (10.31)	21.38 (11.16)
Interval 2	24.97 (17.63)	28.84 (16.40)	20.19 (13.62)	22.87 (16.42)
Interval 3	32.66 (20.51)	25.38 (10.33)	18.56 (15.61)	28.13 (19.72)
Interval 4	26.27 (16.46)	24.82 (14.54)	17.68 (16.89)	23.47 (16.63)
Interval 5	27.11 (17.25)	26.14 (18.16)	20.83 (17.93)	27.82 (22.09)
Interval 6	29.73 (20.67)	[illegible]	24.41 (16.85)	34.28 (21.03)
Interval 7	28.48 (21.34)	30.14 (24.59)	23.41 (19.30)	38.32 (25.70)
Interval 8	33.21 (22.98)	33.30 (22.12)	32.46 (20.21)	37.97 (24.07)
Interval 9	33.89 (24.85)	39.30 (22.27)	24.70 (24.14)	38.90 (24.12)
Interval 10	35.72 (22.97)	42.30 (19.94)	31.27 (23.47)	38.98 (20.41)
Interval 11	33.63 (25.01)	40.52 (20.23)	29.05 (23.75)	40.10 (24.80)
Interval 12	38.26 (21.20)	38.36 (22.35)	33.68 (24.59)	37.20 (22.30)

Note. Parentheses indicate standard deviation.

Margin Distance Traveled

Margin distance traveled is a measurement of the total distance traveled while within a 2-beam margin of the walls of the cage. A 2(Treatment) X 2(Diet) X 12(Time) mixed ANOVA resulted in a significant main effect of margin distance traveled, F (11,639) =

23.01, p = .00, with margin distance traveled decreasing throughout the session (see Table 32).

Table 32. Mean Margin Distance Traveled: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	11.99 (5.89)	11.40 (6.19)	8.82 (5.63)	11.55 (6.54)
Interval 2	8.84 (5.63)	11.08 (5.98)	8.38 (5.70)	8.22 (6.05)
Interval 3	7.28 (4.26)	10.40 (7.40)	5.99 (4.45)	9.27 (6.07)
Interval 4	6.06 (4.35)	6.58 (4.07)	5.53 (4.70)	7.10 (6.63)
Interval 5	5.35 (4.69)	6.11 (3.79)	5.91 (5.68)	7.45 (6.48)
Interval 6	5.07 (4.03)	9.07 (4.36)	6.48 (4.77)	6.39 (4.60)
Interval 7	3.89 (3.69)	3.77 (3.58)	3.61 (4.08)	5.25 (5.88)
Interval 8	3.28 (3.10)	3.87 (3.22)	4.68 (3.49)	3.75 (2.98)
Interval 9	3.35 (2.90)	2.88 (1.98)	2.87 (3.27)	3.75 (3.11)
Interval 10	3.19 (3.54)	4.51 (3.33)	3.60 (3.55)	5.10 (5.81)
Interval 11	2.73 (2.52)	3.77 (2.79)	3.02 (3.12)	5.11 (5.14)
Interval 12	2.87 (2.80)	4.50 (4.20)	4.62 (3.77)	5.61 (3.71)

Note. Parentheses indicate standard deviation.

Stereotypic Movements

Stereotype movements are the total number of XY coordinate changes from, 5 to N, after a .5 to 1 beam coordinate change that fall within + or – 1 beam of the origin of the first movement. It is a measurement of small repetitive behavior that is characteristic of stereotypic movements. A 2 (Treatment) X 2 (Diet) X 12 (Time) mixed ANOVA resulted in a significant main effect of Time, F (11,693) = 7.83, p = .00 with number of

movements slowing increasing through interval 6 and then gradually decreasing during the second half of the session (see Table 33).

Table 33. Mean Stereotypic Movements Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	1.80 (.93)	2.20 (.71)	2.00 (.80)	1.62 (.59)
Interval 2	2.33 (1.09)	2.70 (1.09)	2.71 (1.26)	2.85 (1.06)
Interval 3	2.74 (1.15)	2.62 (.96)	3.22 (1.44)	2.87 (1.06)
Interval 4	2.80 (.99)	3.30 (1.20)	3.06 (1.21)	2.67 (1.44)
Interval 5	2.45 (1.44)	2.52 (.75)	2.87 (1.44)	4.05 (2.53)
Interval 6	2.66 (1.11)	2.96 (.85)	3.51 (1.23)	3.05 (1.89)
Interval 7	2.76 (1.70)	2.42 (1.02)	2.56 (1.46)	3.18 (2.78)
Interval 8	2.03 (1.21)	2.12 (1.01)	2.23 (1.32)	2.30 (1.51)
Interval 9	2.22 (1.41)	1.18 (.80)	2.16 (1.41)	2.15 (1.55)
Interval 10	1.93 (1.32)	1.64 (1.32)	2.13 (1.55)	2.08 (1.90)
Interval 11	1.83 (1.18)	1.94 (1.44)	2.25 (1.82)	2.72 (2.24)
Interval 12	2.27 (1.21)	2.98 (1.65)	2.55 (1.47)	2.58 (1.21)

Note. Parentheses indicate standard deviation.

Stereotypic Time

Stereotypic time is a measurement of the time spent in a series of stereotypic movements separated by at least 10 sample intervals of rest. It is a measurement of time spent in episodes of small, repetitive, stereotypic behavior. A 2(Treatment) X 2(Diet) X 12 (Time) Greenhouse-Geisser corrected ANOVA resulted in significant within-subjects main effect of Time, F (1.28,80.90) = 289.27, p = 00, with most time spent in stereotypic

movement occurring during the first interval and then dropping dramatically to near 0 for the rest of the intervals for all animals (see Table 34).

Table 34. Mean Stereotypic Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	1.95 (.96)	2.82 (.87)	2.22 (.96)	1.87 (.71)
Interval 2	.16 (.23)	.16 (.21)	.15 (.21)	.25 (.27)
Interval 3	.06 (.14)	.06 (.10)	.11 (.16)	.10 (.13)
Interval 4	.07 (.11)	.06 (.10)	.04 (.08)	.13 (.16)
Interval 5	.09 (.17)	.08 (.10)	.07 (.12)	.08 (.13)
Interval 6	.07 (.13)	.06 (.14)	.03 (.07)	.05 (.09)
Interval 7	.03 (.08)	0 (0)	.05 (.13)	0 (0)
Interval 8	.01 (.04)	0 0	.02 (.06)	.02 (.06)
Interval 9	0 (0)	0 (0)	.01 (.04)	0 (0)
Interval 10	0 (0)	0 (0)	0 (0)	0 (0)
Interval 11	0 (0)	0 (0)	.01 (.04)	0 (0)
Interval 12	.04 (.10)	0 (0)	.02 (.09)	.02 (.06)

Note. Parentheses indicate standard deviation.

A significant three-way within-subjects interaction of Time X Treatment X Diet was also found, F (1.28, 80.90) = 6.04, p = .010 (see Table 35). Post-hoc t-test analysis found that the differences were found in first interval with Zinc-deprived/Implant animals spent significantly more time in stereotypic movements (M= 2.82), then Zinc-adequate/Implant animals (M = 1.95). The Zinc-deprived/Implant animals (M= 2.82) also had significantly more time than Zinc-deprived/Sham-surgery animals (M= 1.87) (see Figure 4).

Table 35. Source Table for Mean Stereotypic Time

Source	SS	df	MS	F	Prob.	Power	Eta^2
Interval	254.31	1.28	198.06	289.27	.00*	1.0	.82
Interval X Treatment	1.71	1.28	1.34	1.95	.16	.32	.03
Interval X Diet	1.01	1.28	.79	1.15	.30	.20	.02
Interval X Treatment X Diet	5.31	1.28	4.14	6.04	.01*	.75	.09
Error	55.39	80.90	.69				

Note. *= $p < .05$.

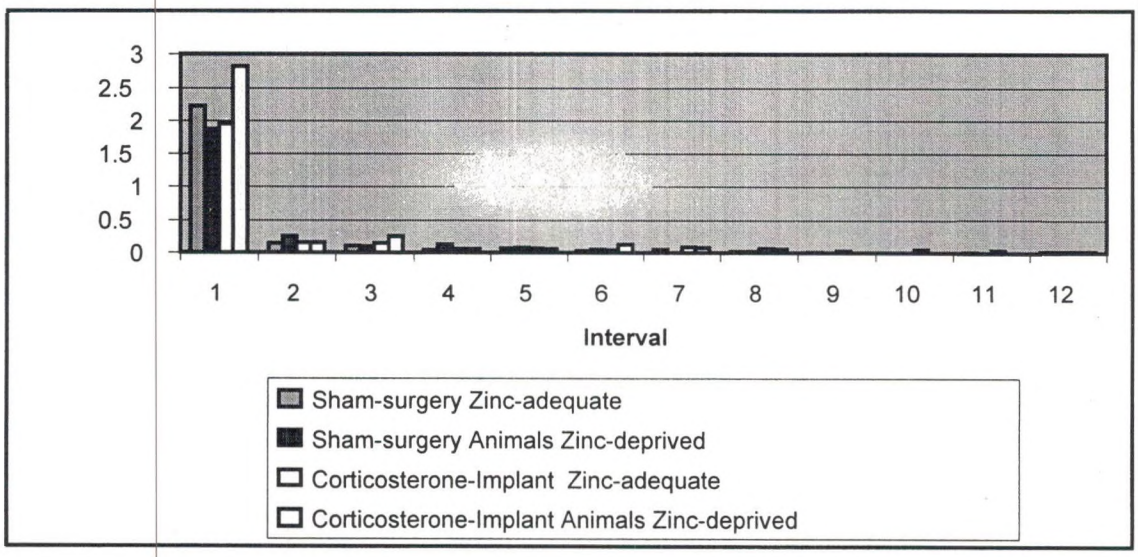

Figure 7. Bar graph showing mean stereotypic episodes divided by treatment on short-term activity.

Vertical Breaks

Vertical breaks are the total number of entries or rearings above the vertical place. Vertical breaks occur when the animal places their head and or paws above a predetermined height. A 2 (Treatment) X 2 (Diet) X 12(Time) mixed ANOVA resulted in a significant main effect of Time, F (11,693) = 11.52, p = .00 with the number of breaks declining through the second half of the session (see Table 36).

Table 36. Mean Vertical Breaks: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	38.33 (15.48)	43.58 (8.44)	32.31 (18.13)	38.63 (18.59)
Interval 2	41.37 (18.22)	44.88 (7.77)	37.37 (19.31)	39.73 (21.82)
Interval 3	39.12 (20.73)	44.18 (10.46)	37.93 (22.24)	40.92 (20.83)
Interval 4	40.88 (18.51)	45.20 (7.98)	37.80 (21.57)	39.98 (21.65)
Interval 5	38.36 (20.47)	43.34 (14.55)	37.27 (22.43)	37.28 (20.66)
Interval 6	35.77 (21.05)	50.34 (5.91)	37.01 (19.70)	32.68 (23.91)
Interval 7	36.36 (20.12)	40.98 (22.88)	31.11 (25.23)	25.68 (26.22)
Interval 8	[illegible]	34.46 (26.10)	30.78 (23.92)	22.42 (25.46)
Interval 9	28.90 (23.43)	30.50 (27.70)	26.67 (22.64)	19.83 (23.52)
Interval 10	27.40 (21.72)	33.62 (23.83)	26.32 (24.15)	15.18 (21.94)
Interval 11	30.72 (23.75)	41.52 (17.32)	32.52 (25.25)	22.50 (25.72)
Interval 12	31.77 (22.58)	34.82 (21.54)	32.40 (23.74)	24.43 (21.66)

Note. Parentheses indicate standard deviation.

Vertical Time

Vertical time is the cumulative that any part of the animal is above the plane of the vertical sensor ring. A 2(Treatment) X 2(Diet) 12 (Time) mixed ANOVA resulted in a significant main effect of Time, F (11,693) = 8.90, p= .00 with time peaking at interval 6 and then dropping through the end of the session (see Table 37).

Table 37. Mean Vertical Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	35.75 (10.77)	38.14 (9.72)	31.45 (13.94)	37.40 (14.13)
Interval 2	41.90 (13.48)	41.40 (7.28)	39.86 (11.91)	40.90 (18.48)
Interval 3	38.97 (16.31)	40.72 (10.34)	43.17 (14.79)	42.92 (16.60)
Interval 4	43.63 (10.70)	42.66 (7.74)	44.05 (13.53)	41.83 (18.74)
Interval 5	40.60 (17.14)	40.94 (14.46)	41.70 (15.29)	40.22 (15.44)
Interval 6	37.99 (16.76)	48.36 (6.88)	42.30 (11.09)	38.35 (19.85)
Interval 7	39.56 (16.90)	40.20 (22.81)	37.02 (20.96)	29.22 (25.88)
Interval 8	39.56 (16.90)	40.20 (22.81)	37.02 (20.96)	29.22 (25.88)
Interval 9	32.97 (19.25)	33.66 (25.85)	36.75 (20.33)	26.25 (25.74)
Interval 10	30.23 (23.01)	30.14 (27.77)	30.10 (21.39)	23.70 (24.38)
Interval 11	28.82 (21.56)	32.84 (24.03)	29.30 (22.98)	18.53 (23.17)
Interval 12	32.62 (21.83)	40.62 (17.34)	34.81 (23.22)	25.38 (25.09)

Note. Parentheses indicate standard deviation.

Front Half Entries

Front half entries is the total number of times the animal entered the front half of the apparatus. A difference in amount of time spent in the front of the apparatus may indicate a difference in anxiety level. A 2(Treatment) X 2(Diet) X 12(Time) mixed ANOVA resulted in a significant main effect of Time, F (11, 693) = 10.80, p = .00 with the number of entries declining after the fifth interval (see table 38).

Table 38. Mean Front Half Entries: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	2.16 (1.18)	2.22 (1.18)	1.82 (.87)	1.67 (1.45)
Interval 2	1.57 (1.43)	.98 (.84)	2.92 (4.65)	1.75 (1.45)
Interval 3	1.52 (1.83)	1.14 (1.07)	1.63 (1.90)	1.15 (1.22)
Interval 4	1.42 (1.97)	1.24 (1.22)	1.45 (1.75)	1.52 (2.07)
Interval 5	1.28 (2.17)	1.26 (1.97)	1.86 (2.53)	.95 (1.58)
Interval 6	.60 (.85)	.34 (.55)	1.61 (2.34)	.37 (.52)
Interval 7	.43 (.67)	.40 (1.06)	1.69 (4.94)	.22 (.42)
Interval 8	.32 (.76)	.30 (.59)	.89 (1.86)	
Interval 9	.30 (.77)	.00 (.00)	.79 (2.19)	.03 (.08)
Interval 10	.43 (.98)	.04 (.13)	.27 (.62)	.42 (.60)
Interval 11	.03 (.13)	.02 (.06)	.48 (1.07)	.23 (.63)
Interval 12	.41 (1.28)	.34 (.43)	.40 (.86)	.08 (.20)

Note. Parentheses indicate standard deviation.

Front Half Time

Front half time is a measurement of the time spent in the front half of the apparatus (see table 39). A 2(Treatment) X 2(Diet) X 12 (Time) Greenhouse-Geisser corrected ANOVA resulted in a significant Time X Diet interaction, F (4.20, 264.26) = 2.93, p = .019 (see table 40). Subsequent t-test (2-tailed) analysis revealed that, for the zinc-adequate animals, more time was spent in the front half of the apparatus during intervals 4 (M = 38.43), 6 (M = 39.42), 7 (M = 41.52), 8 (M= 43.34), 9 (M = 44.71), 10 (M =

43.01), 11 (M = 41.59) and 12 (M = 40.294) than during interval 1 (M = 31.29). For the Zinc-deprived group the animals spent more time in the front part of the apparatus during interval 3 (M = 36.19) than during interval 6 (M = 27.32). None of the other intervals were significantly different.

Table 39. Mean Front Half Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	28.07 (10.43)	35.62 (10.90)	34.51 (11.59)	29.02 (13.90)
Interval 2	31.64 (16.30)	37.10 (18.61)	35.41 (10.99)	32.12 (14.24)
Interval 3	27.13 (20.97)	37.00 (16.58)	39.22 (16.02)	35.38 (18.13)
Interval 4	34.35 (19.45)	31.00 (20.00)	42.51 (14.50)	30.28 (19.89)
Interval 5	30.29 (22.65)	34.66 (22.67)	42.76 (13.07)	25.25 (21.79)
Interval 6	33.83 (24.38)	33.62 (25.05)	45.01 (13.54)	21.02 (25.44)
Interval 7	33.90 (25.51)	36.26 (30.61)	49.14 (15.48)	25.03 (27.56)
Interval 8	37.57 (26.74)	37.24 (28.44)	49.11 (17.24)	24.35 (27.82)
Interval 9	39.42 (25.53)	36.00 (30.98)	50.01 (19.44)	22.48 (28.26)
Interval 10	37.96 (27.35)	35.36 (30.50)	48.06 (22.15)	24.90 (27.24)
Interval 11	38.04 (28.55)	35.44 (30.55)	45.15 (23.40)	24.18 (27.97)
Interval 12	36.26 (27.96)	36.14 (26.24)	44.33 (24.56)	28.62 (28.18)

Note. Parentheses indicate standard deviation.

The zinc-adequate animals also spent more time in the front of the cage than the Zinc-deprived animals during interval 6 (zinc-adequate M = 39.42; Zinc-deprived M = 27.32),

interval 7 (zinc-adequate M = 41.52; Zinc-deprived M = 30.65), interval 8 (zinc-adequate M = 43.34; Zinc-deprived M = 30.80), interval 9 (zinc-adequate M = 44.71; Zinc-deprived M = 29.24), interval 10 (zinc-adequate M = 43.01; Zinc-deprived M = 30.13), interval 11 (zinc-adequate M = 41.59; Zinc-deprived M = 29.81) and interval 12 (zinc-adequate M = 40.29; Zinc-deprived M = 32.38).

Table 40. Source Table for Mean Front Half Time

Source	SS	df	MS	F	Prob.	Power	Eta^2
Interval	1836.38	4.20	437.80	.86	.49	.28	.01
Interval X Treatment	756.48	4.20	180.35	.35	.85	.13	.01
Interval X Diet	6262.78	4.20	1493.06	2.93	.02*	.80	.04
Interval X Treatment X Diet	[illegible]	4.20	369.66	.73	.58	.24	.01
Error	134559.94	264.26	509.20				

Note. * = p <.05

Back Half Entries

Back half entries is the total number of times the animal entered the half of the apparatus that was closest to the wall. This measurement may reflect the amount of anxiety or stress that the animal is experiencing during the session. A 2(Treatment) X 2(Diet) X 12 (Time) mixed ANOVA a significant main effect of back half entries, F (11,693) = 11.37, p = .00, with back half entries declining throughout the session (see Table 41).

Table 41. Mean Back Half Entries: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	2.16 (1.17)	2.14 (1.14)	1.97 (.85)	1.70 (1.58)
Interval 2	1.66 (1.49)	1.02 (.71)	2.79 (4.58)	1.90 (1.56)
Interval 3	1.46 (1.74)	1.20 (1.09)	1.65 (1.89)	1.07 (1.17)
Interval 4	1.48 (2.09)	1.32 (1.27)	1.48 (1.68)	1.45 (1.97)
Interval 5	1.24 (2.22)	1.14 (1.85)	1.85 (2.61)	.95 (1.56)
Interval 6	.63 (.84)	.34 (.54)	1.60 (2.36)	.37 (.53)
Interval 7	.44 (.67)	.34 (.94)	1.59 (4.77)	.20 (.42)
Interval 8	.32 (.73)	.28 (.56)	.89 (1.86)	.37 (.81)
Interval 9	.29 (.74)	0 (0)	.83 (2.25)	.05 (.12)
Interval 10	.41 (.90)	.04 (.13)	.30 (.68)	.38 (.61)
Interval 11	.06 (.21)	.02 (.06)	.47 (1.06)	.23 (.63)
Interval 12	.39 (1.28)	.34 (.44)	.35 (.75)	.08 (.18)

Note. Parentheses indicate standard deviation.

Back Half Time

Back half time is a measurement of the cumulative time spent in the back half of the cage. An animal which spends more time in the back half of the cage may reflect a higher anxiety level (see Table 42). A Greenhouse-Geisser 2 (Treatment) X 2 (Diet) X 12 (Time) mixed ANOVA was calculated due violation of the assumption of sphericity as determined by Mauchly's Test of Sphericity $W = .002$, $p = .000$.

Table 42. Mean Back Half Time: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	31.93 (10.43)	24.38 (10.90)	25.50 (11.59)	30.98 (13.90)
Interval 2	28.36 (16.30)	22.90 (18.61)	24.59 (10.99)	27.88 (14.24)
Interval 3	32.87 (20.97)	23.00 (16.58)	20.78 (16.02)	24.62 (18.13)
Interval 4	25.65 (19.45)	29.00 (20.90)	17.49 (14.50)	29.72 (19.89)
Interval 5	29.71 (22.65)	25.34 (22.67)	17.24 (13.07)	34.75 (21.79)
Interval 6	26.17 (24.38)	26.38 (24.05)	14.99 (13.54)	38.98 (25.44)
Interval 7	26.10 (25.51)	23.74 (30.61)	10.86 (15.48)	34.97 (27.56)
Interval 8	22.43 (26.74)	22.76 (28.44)	10.89 (17.24)	[illegible]
Interval 9	20.58 (25.53)	24.00 (30.98)	9.99 (19.44)	37.52 (28.26)
Interval 10	22.04 (27.35)	24.64 (30.50)	11.94 (22.15)	35.10 (27.24)
Interval 11	21.96 (28.55)	24.56 (30.55)	14.85 (23.40)	35.82 (27.97)
Interval 12	23.74 (27.96)	23.86 (26.24)	15.67 (24.56)	31.38 (28.18)

Note. Parentheses indicate standard deviation.

A significant Time X Diet interaction was found, F (4.20, 264.26) = 2.93, p = .02. Post-hoc analysis resulted in a significant difference between diet for intervals 6,(Zinc-adequate M= 20.58; Zinc-deprived M= 32.68), interval 8 (Zinc-adequate M= 16.66; Zinc-deprived M= 29.21) and interval 9 (Zinc-adequate M= 15.29; Zinc-deprived M= 30.76)with Zinc-deprived animals spending more time in the back half of the cage than zinc-adequate rats (see Table 43 and Figure 5).

Table 43. Source Table for Mean Back Half Time

Source	SS	df	MS	F	Prob.	Power	Eta^2
Interval	1836.38	4.20	437.80	.86	.49	.28	.01
Interval X Treatment	756.48	4.20	180.35	.35	.85	.13	.01
Interval X Diet	6262.78	4.20	1493.06	2.93	.02*	.80	.04
Interval X Treatment X Diet	1550.57	4.20	369.66	.73	.58	.24	.01
Error	134559.94	264.26	509.20				

Note. * = p <.05.

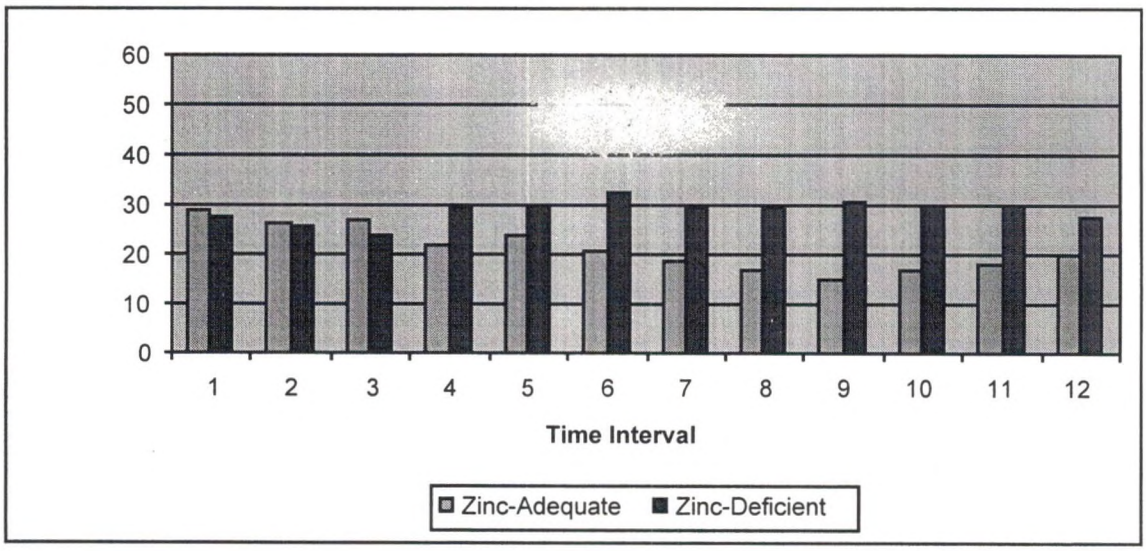

Figure 8. Bar graph showing mean time spent in back half on short-term activity.

Clockwise Revolutions

Clockwise rotations is the total number of clockwise , 360° angular direction changes about the cage center. Differences found in circling behavior as measured with clockwise and counter-clockwise movements may be indicative of brain damage. A 2 (Treatment) X 2 (Diet) X 12(Time) mixed ANOVA resulted in a significant main effect of Time, F (11,

693) = 16.393, p = .00 with the number of revolutions dropping off after interval 3 to a very low frequency for the rest of the session (see Table 44).

Table 44. Mean Clockwise Revolutions Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	.28 (.27)	.22 (.20)	.22 (.29)	.17 (.25)
Interval 2	.13 (.14)	.08 (.14)	.18 (.19)	.05 (.12)
Interval 3	.08 (.14)	.10 (.19)	.16 (.20)	.08 (.13)
Interval 4	.06 (.11)	.04 (.08)	.10 (.15)	.07 (.18)
Interval 5	.03 (.09)	.02 (.06)	.15 (.22)	.03 (.08)
Interval 6	.02 (.06)	0 (0)	.08 (.13)	0 (0)
Interval 7	.03 (.07)	0 (0)	0 (0)	0 (0)
Interval 8	.02 (.06)	0 (0)	.04 (.10)	.03 (.12)
Interval 9	.03 (.10)	0 (0)	.02 (.06)	.02 (.06)
Interval 10	0 (0)	0 (0)	.01 (.04)	.03 (.08)
Interval 11	0 (0)	0 (0)	.01 (.04)	.03 (.12)
Interval 12	.01 (.04)	.02 (.06)	0 (0)	.03 (.08)

Note. Parentheses indicate standard deviation.

Counter-Clockwise Revolutions

Counter-clockwise revolutions is a measurement of the total number of counter-clockwise, 360° angular direction changes. A 2 (Treatment) X 2 (Diet) X 12 (Time) mixed ANOVA resulted in a significant main effect of Time, F (11,693)= 25.56, p = .000

with frequency of revolutions dropping to near 0 after interval 3 (see Table 45).

Table 45. Mean Counter- Clockwise Revolutions: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Interval 1	.37 (.29)	.18 (.22)	.21 (.27)	.35 (.33)
Interval 2	.16 (.23)	.16 (.21)	.15 (.21)	.25 (.27)
Interval 3	.06 (.14)	.06 (.10)	.11 (.16)	.10 (.13)
Interval 4	.07 (.11)	.06 (.10)	.04 (.08)	.13 (.16)
Interval 5	.09 (.17)	.08 (.10)	.07 (.12)	.08 (.14)
Interval 6	.07 (.13)	.06 (.14)	.03 (.07)	.05 (.09)
Interval 7	.03 (.08)	0 (0)	.05 (.13)	0 (0)
Interval 8	.01 (.04)	0 (0)	.02 (.06)	.02 (.06)
Interval 9	0 (0)	0 (0)	.01 (.04)	0 (0)
Interval 10	0 (0)	0 (0)	0 (0)	0 (0)
Interval 11	0 (0)	0 (0)	.01 (.04)	0 (0)
Interval 12	.04 (.10)	0 (0)	.02 (.09)	.02 (.05)

Note. Parentheses indicate standard deviation.

Analysis of Interval 7: First Sound Interval

The first interval in which the 1500 Hz tone was sounded was analyzed for the effects of the stressor on the activity measures (see Table 46). A 2(Treatment) X 2 (Diet) ANOVA for the 18 variables resulted in a significant difference for front half time for the interaction of Treatment X Dietary group F (1,63)= 4.49, p =.04.

Table 46. Mean Activity Measurements for Interval 7: 1st Sound Interval

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
XY Movement	8.08 (3.09)	7.70 (3.40)	8.44 (3.61)	6.88 (3.27)
XY Movement Time	21.42 (10.62)	18.12 (10.17)	23.05 (12.38)	17.62 (10.78)
XY Rest Time	38.58 (10.62)	41.88 (10.17)	36.95 (12.38)	42.38 (10.78)
XY Distance Traveled	16.97 (9.93)	13.04 (8.62)	19.93 (14.05)	12.63 (8.62)
Stereotype Movements	2.28 (1.36)	1.96 (.90)	2.09 (1.27)	2.65 (2.03)
Stereotype Time	2.76 (1.70)	2.42 (1.02)	2.56 (1.46)	3.18 (2.78)
Counter-Clockwise R.	.03 (.08)	0 (0)	.05 (.13)	0 (0)
Clockwise Revolutions	.03 (.07)	0 (0)	0 (0)	
Margin Distance	3.89 (3.69)	3.77 (3.58)	3.61 (4.08)	5.25 (5.88)
Margin Time	28.48 (21.34)	30.14 (24.59)	23.41 (19.30)	38.32 (25.70)
Front Half Time	33.90 (25.51)	36.26 (30.61)	49.14 (15.48)	25.03 (27.56)
Front Half Entries	.43 (.67)	.40 (1.06)	1.69 (4.94)	.22 (.42)
Back Half Time	30.20 (25.96)	19.30 (26.31)	22.94 (24.59)	21.02 (28.91)
Back Half Entries	1.43 (3.81)	.14 (.33)	1.27 (2.38)	.03 (.08)
Vertical Breaks	36.36 (20.12)	40.98 (22.88)	31.11 (25.23)	25.68 (26.22)
Vertical Time	39.56 (16.90)	40.20 (22.81)	37.02 (20.96)	29.22 (25.88)
Nose Poke Entries	.93 (1.93)	.86 (1.07)	1.79 (3.03)	1.03 (1.90)
Nose Poke Time	2.43 (4.95)	.88 (1.49)	2.17 (4.03)	2.48 (4.37)

Note. Parentheses indicate standard deviation.

Subsequent t-test analysis (2-tailed) resulted in a significant difference for Sham surgery animals with a zinc-adequate diet, with sham surgery animals (M = 49.14) spending more time in the front half of cage than Zinc-deprived animals, sham surgery animals (M = 25.03).

Total Session Activity

To calculate total activity for the entire session, front half entries and back half entries for all 12 intervals during the 60 minute session was summed together to create a total activity variable. A 2 (Treatment) X 2 (Diet) ANOVA resulted in no significant differences (see Table 47).

Table 47. Mean Total Session Activity: Treatment X Diet

	Treatment = Implant	Treatment= Sham Surgery
Zinc- Adequate	21.01 (14.33)	31.61 (36.87)
Zinc-deprived	16.46 (12.13)	17.50 (12.18)

Note. Parentheses indicate standard deviation.

Nose-Poke Task

Prior to the short-term activity monitoring task, the 16 holes in the floor were baited with a sucrose reinforcement pellet. The Tru-Scan Activity Monitoring program (Coulbourn Instruments) measured several variables related to the consumption of the food pellets by the animals. Task latency is the time from the start of the session until the animal enters the first hole. Task time is the time from the entry into the first hole until the animal has entered all 16 holes at least once.

Task entries is the total number of entries in all holes prior to completing the task. Post-task entries is the total number of entries into all holes after task completion. Novel

hole inter-response-time (IRT) is the average time from one novel hole entry to the next novel hole entry. Repeats between novels is the total number of hole re-entries between novel hole entries. The number of pellets consumed by each animal during the session was also recorded. A 2 (Treatment) X 2 (Diet) ANOVA resulted in no significant differences for any of these variables (see Table 48).

Table 48. Mean Nose Poke Task: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Task Latency	85.22 (134.05)	59.30 (55.37)	68.29 (57.16)	33.42 (29.91)
Task Time	1919.48 (1395.76)	1949.70 (1423.33)	2186.21 (1263.93)	1870.25 (1383.68)
Novel Hole IRT	151.00 (141.52)	96.99 (89.85)	122.66 (80.77)	232.70 (465.45)
Task Entries	40.65 (22.46)	55.80 (14.01)	56.04 (26.81)	48.83 (36.40)
Post-task Entries	37.04 (45.53)	27.10 (33.88)	30.17 (37.91)	44.25 (56.82)
Repeat Between Novels	26.17 (18.34)	36.10 (13.88)	37.12 (25.59)	29.17 (26.71)
Pellets Consumed	9.91 (7.77)	9.60 (8.26)	12.68 (6.23)	10.67 (7.88)

Note. Parentheses indicate standard deviation.

Physiological Measurements during Short-Term Activity Monitoring

After each session, the occurrence of defecatation and urination was recorded on the log. These measures may indicate the amount of anxiety or stress the animal experienced during the short-term activity session. Frequency for the occurrence of each measurement and non-parametric Chi-square test results are presented in tables 49 and

50. Only dietary group was significantly different on frequency of urination with zinc-adequate animals having a higher frequency than Zinc-deprived animals.

Table 49. Physiological Measurements: Frequencies and Significance Tests by Diet

Group	Zinc -adequate	Zinc-deprived	X^2	p
Defecation	11	4	3.27	.07
Urination	27	8	10.31	.00

Table 50. Physiological Measurements: Frequencies and Significance Tests by Treatment

Group	Corticosterone Implant	Sham Surgery	X^2	p
Defecation	7	8	.07	.80
Urination	17	18	.03	.87

Radial Arm Maze

The number of pellets consumed during the training sessions and the two experimental sessions were recorded. The mean was calculated for the last three training sessions and this made up the baseline measurement. The mean for all three sessions during each of the two experimental sessions (session 1: week 3; session 2: week 11) was also calculated. Those animals which had a mean of 0 or 1 pellet consumed during the last three training sessions were excluded from further analysis due to a failure to learn/try the task (N=31 animals) (see table 51). A 2 (Treatment) X 2 (Diet) ANOVA on the baseline data resulted in no significant differences between groups prior to treatment.

Table 51. Mean Pellets Consumed in Radial Arm Maze: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Baseline	5.07 (1.62)	3.67 (1.73)	4.35 (2.07)	5.58 (1.27)
Session 1 (baseline covariate)	1.13 (2.13)	0 (0)	.49 (.93)	1.29 (1.56)
Session2 (baseline covariate)	1.27 (2.55)	.22 (.38)	.09 (.20)	1.00 (1.62)

Note. Parentheses indicate standard deviations.

A 2 (Treatment) X 2 (Diet) X 2 (Time) mixed ANOVA of the session 1 and session 2 means also resulted in no significant differences. A 2 (Treatment) X 2 (Diet) X 2(Time) mixed ANCOVA on the session 1 and session 2 means with the baseline means as a covariate also resulted in no significant difference. An examination of the mean pellets consumed suggests that a floor effect may be affecting analysis, as the number of pellets consumed was very small across groups.

Table 52. Mean Square Root Transformed Pellets Consumed in Radial Arm Maze: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Baseline	2.22 (.41)	1.87 (.50)	2.02 (.53)	2.35 (.28)
Session 1 (baseline covariate)	.62 (.91)	.00 (00)	.46 (.55)	.90 (.74)
Session2 (baseline covariate)	.57 (1.02)	.27 (.47)	.13 (.28)	.64 (.82)

Note. Parentheses indicate standard deviations.

The data was then transformed using a square root transformation to control for this possible floor effect and heterogeneous variance (see table 52). A 2 (Treatment) X 2(Diet) ANOVA on the baseline means resulted in no significant differences. A 2(Treatment) X 2(Diet) X 2(Time) mixed ANOVA for the session 1 and session 2 means resulted in no significant differences. A 2(Treatment) X 2(Diet) X 2(Time) mixed ANCOVA for the session 1 and session 2 means with the baseline measure as the covariate resulted in a significant Time X Treatment interaction, $\underline{F}$ (1, 31) = 4.71, $\underline{p}$ = .038. Post-hoc independent t-tests (2-tailed) resulted in a significant difference on session 1 with sham surgery rats ($\underline{M}$ = .70) consuming significantly more pellets than corticosterone implanted rats ($\underline{M}$ = .33). The sham surgery rats during session one ($\underline{M}$ = .67) also consumed significantly more pellets than during session two ($\underline{M}$ = .35) (see table 53).

Table 53. ANCOVA Source Table for Square Root Transformed Pellets Consumed in Radial Arm Maze

Source	SS	df	MS	F	Prob.	Power	Eta^2
Time	.57	1	.57	3.85	.06	.48	.11
Time X Baseline	.49	1	.49	3.31	.08	.42	.10
Time X Treatment	.70	1	.70	4.71	.04*	.56	.13
Time X Diet	.14	1	.14	.93	.34	.16	.03
Time X Treatment X Diet	.19	1	.19	1.26	.27	.19	.04
Error	4.62	31	.15				

Note. * = $p < .05$.

During each session the Coulbourn Instruments computer software "L2T2S" ran the radial arm maze including the opening and closing of guillotine doors. The software also

recorded each time the animal entered one of the eight arms or entered the feeder by counting a break in an infra-red beam. For each of the three trials on each day, the total number of entries into all eight feeders was determined and used for analysis. Also, the mean number of entries for all three trials in a day was computed and used to compare session one with session two.

Table 54. Mean Feeder Entries for Session One Radial Arm Maze: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-Adequate	Zinc-deprived	Zinc-Adequate	Zinc-deprived
Trial 1	4.70 (5.62)	0 (0)	1.47 (1.85)	7.63 (6.26)
Trial 2	5.20 (8.70)	1.67 (2.89)	1.80 (2.57)	13.75 (12.86)
Trial 3	5.60 (11.15)	.67 (1.15)	2.20 (3.88)	12.25 (17.66)

Note. Parentheses indicate standard deviations.

Means and standard deviations for session one are presented in table 54. For session one, a 2 (Treatment) X 2(Diet) X 3(Time: 3 trials) mixed ANOVA resulted in a significant interaction of Treatment X Diet, F (1, 32) = 6.21, p = .018.

Table 55. ANOVA Source Table for Feeder Entries for Session One Radial Arm Maze

Source	SS	df	MS	F	Prob.	Power	Eta^2
Intercept	1728.24	1	1728.24	11.78	.00	.91	.27
Treatment	241.02	1	241.02	1.64	.21	.24	.05
Diet	119.87	1	119.87	.82	.37	.14	.03
Treatment X Diet	910.80	1	910.80	6.21	.02*	.68	.16
Error	4696.93	32	146.78				

Note. * = p <.05.

Subsequent analysis demonstrated that the sham-surgery, Zinc-deprived animals (M = 11.21) had significantly more feeder entries than the sham surgery, zinc-adequate animals (M = 1.822), and the corticosterone implant, Zinc-deprived animals (M = .778). No within subjects significant effects were found (see table 55).

Table 56. Mean Feeder Entries for Session Two Radial Arm Maze: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Trial 1	7.20 (13.69)	1.33 (1.53)	1.00 (1.62)	8.38 (10.91)
Trial 2	7.20 (13.69)	1.33 (1.53)	1.00 (1.62)	8.38 (10.91)
Trial 3	7.50 (13.79)	.33 (.58)	1.43 (2.93)	3.63 (9.07)

Note. Parentheses indicate standard deviations.

Table 57. Source Table for Feeder Entries for Session Two Radial Arm Maze

Source	SS	df	MS	F	Prob.	Power	Eta^2
Time	26.69	2	13.35	1.92	.15	.38	.06
Time X Treatment	13.88	2	6.94	1.00	.38	.22	.03
Time X Diet	44.43	2	22.22	3.19	.05*	.59	.09
Time X Treatment X Diet	15.93	2	7.96	1.14	.33	.24	.04
Error	431.35	62	6.96				

Note. * = $p < .05$.

Means and standard deviations for session two are presented in table 56. For session two, a 2(Treatment) X 2(Diet) X 3(Time: 3 trials) mixed ANOVA resulted in a significant Time X Diet interaction, $F(2,62) = 3.19$, $p = .048$. Subsequent analysis

resulted in the Zinc-deprived animals entering the pellet feeder (M = 1.98) significantly less on trial three than during trial one (M = 4.85) and trial two (M= 4.85). The zinc deficient animals during trial three (M = 1.98) also entered the pellet feeder significantly less than the zinc-adequate animals during trial one (M = 4.10), trial two (M = 4.10) and trial three (M = 4.46). No other significant effects were found (see Table 57).

Table 58. Mean Feeder Entries Radial Arm Maze: Session X Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Session One	5.17 (8.30)	.78 (1.35)	1.87 (2.39)	11.21 (11.16)
Session Two	7.30 (13.54)	1.00 (1.00)	1.21 (2.07)	6.79 (9.69)

Note. Parentheses indicate standard deviations.

The mean for all three trials for each of the two sessions was calculated and is presented in table 58. A 2(Treatment) X 2(Diet) X 2 (Time: session one and two) mixed ANOVA resulted in a significant between subjects Treatment X Diet difference, F (1, 30) = 4.80, p = .04.

Table 59. Source Table for Mean Feeder Entries Radial Arm Maze: Session X Treatment X Diet

Source	SS	df	MS	F	Prob.	Power	Eta^2
Intercept	981.97	1	981.97	9.12	.01	.83	.23
Treatment	36.74	1	36.74	.34	.56	.09	.01
Diet	14.11	1	14.11	.13	.72	.06	.00
Treatment X Diet	516.31	1	516.31	4.80	.04*	.56	.14
Error	3229.52	30	107.65				

Note. * = $p < .05$.

Subsequent analysis resulted in the sham-surgery, Zinc-deprived animals (M = 9.00) entering the pellet feeders significantly more times than the corticosterone-implanted, Zinc-deprived animals (M = .89). No other significant effects were found (see table 59).

Water Maze

Water maze testing occurred in two sessions. Session one was during week five and session two was during week 12. During each trial, the latency to complete the maze and the amount of boli (defecation) were recorded. If an animal failed to complete the maze a latency of 300 seconds was recorded. The video-taped trials were then scored for number of errors. An error was recorded when the animal deviated from the path leading to the end of the maze, by going through an entryway leading to a dead-end area. The last three training trial latencies was used as a baseline measure. All three trials for the baseline, session one and session two were averaged providing a single mean for each animal for each session for latency. The three trials for session one and session two were averaged for errors and amount of defecation and a single mean used for each of these measurements. The number of trials required during training to reach criteria (2 consecutive trials under 100 seconds) were also used in this analysis. A 2(Treatment) X 2(Diet) ANOVA on the number of trials required to reach criteria resulted in no significant differences.

A 2 (Treatment) X 2(Diet) X 3 (Time; Baseline, Session one, Session two) on the latency to finish the maze resulted in a significant main effect of Time, F(2, 100) = 184.52, p =.00 (see Table 60). Subsequent analysis revealed that the average latency to finish was significantly less during the baseline trials (M = 119.46) than during Session one (M = 276.36) and Session two (M = 270.66). A significant effect of treatment was

also found, F (1, 50) = 4.53, p = .038. Subsequent analysis revealed that the corticosterone-implant animals had a longer latency to complete the maze (M = 236.88) than the sham-surgery animals (M = 207.44) (see table 61).

Table 60. Mean Water Maze Latency: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Baseline	130.60 (24.94)	124.48 (21.83)	111.13 (21.19)	111.63 (23.98)
Session One	278.36 (39.08)	300.00 (0)	249.60 (90.75)	277.50 (51.07)
Session Two	290.76 (35.80)	297.07 (6.42)	241.27 (112.82)	253.53 (89.62)

Note. Parentheses indicate standard deviations.

Table 61. Source Table for Water Maze Latency

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	31716.16	1	31716.16	4.53	.04*	.55	.08
Diet	3973.87	1	3973.87	.57	.46	.11	.01
Treatment X Diet	360.28	1	360.28	.05	.82	.06	.00
Error	350448.36	50	7008.97				

Note. * = p <.05.

A 2(Treatment) X 2(Diet) X 2(Time) mixed ANOVA on the error measurement revealed a significant main effect of Time, F(1, 48) = 29.18, p = .00. Subsequent analysis revealed that there were significantly more errors during session one (M = 2.68) than session two (M = 1.25) (see Table 62).

Table 62. Mean Water Maze Errors: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Session One	3.16 (3.05)	2.19 (.60)	2.72 (2.30)	2.67 (2.31)
Session Two	1.87 (1.96)	.93 (.81)	.72 (.59)	1.47 (.69)

Note. Parentheses indicate standard deviations.

Table 63. Mean Number of Boli: Treatment X Diet

	Treatment= Implant		Treatment= Sham Surgery	
	Zinc-adequate	Zinc-deprived	Zinc-adequate	Zinc-deprived
Session One	1.07 (.65)	1.37 (.53)	1.08 (.60)	1.23 (.79)
Session Two	1.20 (.52)	1.57 (.57)	1.20 (.63)	1.60 (.70)

Note. Parentheses indicate standard deviations.

Table 64. Source Table for Water Maze Boli

Source	SS	df	MS	F	Prob.	Power	Eta^2
Treatment	.08	1	.08	.15	.70	.07	.00
Diet	2.81	1	2.81	5.51	.02*	.63	.09
Treatment X Diet	.10	1	.10	.20	.66	.07	.00
Error	25.50	50	.51				

Note. * = $p < .05$.

A 2 (Treatment) X 2(Diet) X 2(Time) mixed ANOVA on the number of boli revealed a significant main effect of Time, F (1, 50) = 4.61, p = .037 with significantly more boli during session two (M = 1.42) than during session one (M = 1.20). A significant effect of diet was also found, F (1, 50) = 5.51, p = .02. Subsequent analysis revealed that the

Zinc-deprived animals (M = 1.48) had significantly more defecation than the Zinc-adequate animals (M = 1.14) (see Tables 63 and 64).

Relationship between Behavioral, Blood and Brain Variables

All behavioral variables which yielded significant treatment, diet or interaction differences were analyzed in association with the four measurements of corticosterone concentration, the width measurement of the pyramidal cell band in the CA3 and CA1 areas of the hippocampus and the measurement of zinc concentration in the hilus, CA1 and CA3 areas of the hippocampus, the cortex and the thalamus. Bivariate correlations were run on each variable and significance was determined at the $p = .01$ level. Only those correlations described are those associations that were $p < .01$.

Several correlations were found for the measurement of long-term activity. For session one, total small movement a positive relationship was found with the XY distance traveled during interval 7 of the Short-term activity monitoring $R(50) = .406$. A negative relationship was found for the total small movement measure for session two and the measurement of zinc concentration in the CA3 area of the hippocampus $R(36) = -.448$.

Several correlations were found for the short-term activity measure. A positive association was found between the baseline measurement of plasma corticosterone concentration and the stereotypic movement during the first interval, $R(25) = .578$. The total activity during the short-term activity task was negatively associated with the baseline corticosterone measurement $R(25) = -.664$. The number of pellets consumed during the task was positively associated with the zinc concentration of the CA3 area of the hippocampus, $R(.35) = .453$ and with the measurement of total activity, $R(67) = .403$. Clockwise movement during the entire short-term activity task was positively associated

with the measurement of defecation, R(67) = .311 and the measurement of XY distance during interval 7, R(67) = .509. Counter-clockwise movement during interval 7 was negatively associated with the zinc concentration in the cortex, R(35) = -.615 and positively associated with the XY distance moved during interval 7, R(67) = .509. Counter-clockwise movement during interval 7 was also positively associated with the latency to complete the water maze during session one, R(54) = .358. The incidence of urination during the short-term activity task was positively associated with the latency on the water maze task during session two, R(53) = .382. The total activity measurement was also positively associated with the measurement of total platelets in the blood, R(27) = .584.

Several correlations were found for the radial arm maze. Negative associations were found for the corticosterone plasma concentrations and the number of pellets consumed in the radial arm maze. Session one pellets consumed was negatively associated with the baseline corticosterone measurement, R(27) = -.510 as was the session two pellets consumed, R(27) = -.664. The number of feeders entered during session two was also negatively associated with the baseline corticosterone measurement, R(27) = -.676. The number of pellets consumed during session two was positively associated with the width of the CA3 area of the hippocampus, R(12) = .766. The measurement of the zinc concentration of the CA3 area of the hippocampus was also positively associated with the number of pellets consumed during session one, R(36) = .440 during session two, R(36) = .494. Pellets consumed was also positively associated with the amount of time spent in the back half during interval 6 of the short-term activity monitoring during session one, R(67) = .330 and session two, R(66) = .317.

Several correlations were found for the water maze task. The number of errors during session one was positively associated with the zinc concentration of the CA1 of the hippocampus, R(29) = .473 and the zinc concentration of the CA3 area of the hippocampus, R(29) = .531. The latency to complete the maze was negatively associated with the session one boli count during session one R(54) = -.391 and during session two, R(52) = -.469. The session one boli count was also negatively associated with the number of errors during session one of the water maze task, R(54) = -.361. The boli count during session two was negatively associated with the latency for session two, R(52) = -.487. The number of errors during session two was negatively associated with the latency for session one, R(51) = -.392 and latency for session two, R(53) = -.654. The percentage of neutrophils in the blood was positively associated with the number of errors during session two, R(23) = .532. The latency of session two was positively associated with the hematocrit measurement of the blood, R(21) = .549.

Several correlations were also found for the brain and blood measurements. The zinc concentration of the thalamus was positively correlated with the two month corticosterone plasma concentration, R(25) = .535. The zinc concentration of the CA3 area of the hippocampus was positively associated with the zinc concentration of the hilus area of the hippocampus R(36) = .532 and the CA1 area of the hippocampus, R(36) = .557. The zinc concentration in the thalamus was positively associated with the percentage of eosinophils in the blood, R(12) = .771. The mean cell hemoglobin was positively associated with the corticosterone plasma measurement at one month, R(23) = .636. Mean cell hemoglobin was also positively associated with the percentage of monophils in the blood, R(22) = .679.

CHAPTER IV

DISCUSSION

Conclusions Drawn on Hypothesis One

The first hypothesis of this study was that hippocampal degeneration as a result of long-term GC exposure and ZnD would result in decreased long and short-term spatial memory in Sprague-Dawley rats with a greater decrease in memory in ZnD rats. Hypothesis one was tested using a short-term memory task, the radial arm maze and a long-term memory task, the water maze.

Radial Arm Maze Task

Comparison of the corticosterone implanted animals with the sham-surgery animals resulted in the sham-surgery animals consuming more pellets than the corticosterone-implanted animals during session one of testing with the radial arm maze, which occurred after one month of treatment. This may reflect a difference in short-term memory during the first session. However, all results in the radial arm maze will be difficult to interpret as the task suffered from a floor effect with the animals responding very little overall with few pellets consumed and a small number of feeder entries.

Comparison of the dietary groups resulted in the finding that the Zinc-deprived animals consumed less pellets during session two (week 11- third trial of the radial arm maze) than during the first two trials and less than the zinc-adequate animals during all three trials during session two of testing with the radial arm maze. This may reflect a

difference in short-term memory after eight weeks of dietary treatment with the Zinc-deprived animals having an impaired memory.

An examination of the interaction of corticosterone and zinc-deprivation on the radial arm maze resulted in a compounding of the corticosterone treatment with the dietary treatment over time. During both sessions the Zinc-deprived animals with sham-surgery entered more feeders than the implant, Zinc-deprived animals, but during the first session the sham-surgery, zinc deficient animals out-performed both the sham-surgery, zinc-adequate animals and the corticosterone implant, Zinc-deprived animals.

These mixed results for the interaction overall reflect that a decline in performance was found for the implanted animals in comparison to the sham-surgery animals, supporting the hypothesis. However, the addition of dietary group demonstrated that a decline in overall performance was not found in the Zinc-deprived animals over the zinc-adequate animals and in fact during session one the zinc deficient animals performed better than the adequate animals.

Water Maze Task

Corticosterone treatment resulted in a longer latency to finish the maze for the corticosterone-implanted group as compared to the sham-surgery group. This would reflect a deficit in long-term memory for the animals that received corticosterone treatment at both testing sessions (Session one: five weeks and Session two: 12 weeks).

An examination of the effects of dietary treatment resulted in the Zinc-deprived group excreting more boli than the zinc-adequate animals. This may reflect a difference in anxiety level with the Zinc-deprived animals having a higher level of anxiety.

There were no significant treatment X dietary interactions in the analysis of the water maze data. The results in general reflect a deficit in long-term memory for the corticosterone-implanted animals with no memory deficits found attributable to diet. The Zinc-deprived animals did however show more symptom of anxiety than the zinc-adequate animals (increased boli). These results showed support for the hypothesis only for the comparison of corticosterone treatment.

Conclusions drawn on Hypothesis Two

The second hypothesis of this study was that hippocampal degeneration as a result of long-term GC exposure and ZnD would result in degeneration of the dendrites and cell bodies of the hippocampus, would be apparent in the GC treated animals with more degeneration exhibited in the ZnD rats, and this would correlate to greater deficits found in memory than sham-surgery and zinc-adequate animals. This hypothesis was tested by examination of the general histology of the hippocampus and examination of the zinc concentration in the hippocampus, cortex and thalamus. This hypothesis was also tested by correlating the memory task results with the histology results to see if any association between them would be found.

Histology of the Brain

The histological examination of the hippocampus resulted in the sham-surgery group having a greater pyramidal cell layer width in the CA3 area than the corticosterone-implant animals. This demonstrates a degeneration of the cells in the hippocampus in the corticosterone-implanted animals and is in agreement with Sapolsky (1985). This finding also supported hypothesis two. However, absence of any

deleterious effect of zinc on brain tissues, fails to support the interaction portion of hypothesis two.

The histological measurement of zinc-concentration in the brain resulted in the Zinc-deprived animals having a greater amount of zinc in the vesicles of the cortex than the zinc-adequate animals. This may reflect an attempt at conservation of zinc by the brain in those animals receiving the Zinc-deficient diet.

For the measurement of zinc concentration in brain tissues, only the cortex had a significant interaction with the sham-surgery animals consuming the Zinc-deficient diet having a greater concentration than the animals consuming the zinc-adequate animals. This appeared to be slightly modified by corticosterone treatment in that the animals with an implant and a zinc-adequate [illegible] greater concentration than the sham-surgery animals on a zinc-adequate diet.

Brain and Memory Correlations

For the radial arm maze, a strong association was found between consumption of pellets during session two and the width of the CA3 area leading to the conclusion that short-term memory performance was related to pyramidal cell layer width in the CA3 area of the hippocampus. This association supports the theory that this area may be important in spatial memory. The zinc concentration of the CA3 area was also correlated with the number of pellets consumed during both session one and session two leading to the conclusion that an increase in zinc concentration in this area is related to an increase short-term spatial memory.

For the water maze task, the number of errors was correlated with the zinc concentration in the CA1 and CA3 areas of the hippocampus leading to the conclusion

that an increase in errors was associated with increased zinc concentration in these areas of the hippocampus. It is interesting to note that the only significant dietary effects found on the water maze were differences in symptoms of anxiety. These results support hypothesis two in that some association between memory and brain histology was found.

Conclusions drawn on Activity Measurement

In addition to the findings that were hypothesis driven, many other results were found in this study. Measurements of activity were included in the present study because they have been used as an animal model of depression (Pare, 1994). High levels of GC have been found to be associated with chronic depression (Sheline et al., 1996) and with PTSD (Bremner, 1999). Decreased generalized motor activity in an open-field, short-term activity apparatus has also been found in zinc-deprived rats (Gordon, 1984). In the present study, two activity tasks were done. The first was a long-term activity measurement designed to test the effects of the various treatments early and late in the study. The second task was a short-term activity task and designed to give a more complete study of the effects of treatment mid-way through the study than the long-term activity task.

Long-term Activity Measurement

For the long-term activity task, the animals treated with corticosterone (both diets) had more small movements during session one than during session two. For session two, the implanted, zinc-adequate animals had more small movements than the implanted, Zinc-deprived animals. For the sham-surgery, animals the zinc-adequate animals had more small movements than the Zinc-deprived animals. The appearance of a significant difference early in the study for the corticosterone treatment leads to the conclusion that

the effects of corticosterone on activity occurs early (within the first month) and is then compounded later in the study by zinc-deprivation with a decrease in movement found in the animals receiving both treatments.

Short-Term Activity Measurement

The short-term activity task during week eight resulted in several dietary differences. The Zinc-deprived animals spent more time in the back of the apparatus during the second half of the session when the stressor noise was sounded reflecting a possible difference in anxiety levels. They also had less clockwise movement indicating the possibility of brain damage (Penland, 2002). The Zinc-deprived animals also had less urination than the zinc-adequate animals indicating a potential difference in level of anxiety. In particular, during the interval containing the first stressor noise, the Zinc-deprived animals had less counter-clockwise movement and XY distance movement than the zinc-adequate animals indicating a decrease in activity level.

Examination of the interaction between corticosterone and zinc-deprivation, the implanted, Zinc-deprived animals spent more time in stereotypic movements than either the implant/zinc-adequate or the sham-surgery/Zinc-deprived animals. The increase in stereotypic movements may be indicative of brain damage and it is interesting that this result occurred in the animals that received both treatment manipulations. During interval 7 when the first noise stressor occurred, the sham surgery/Zinc-adequate animals spent more time in the front half of the apparatus than the sham-surgery/Zinc-deprived animals lending support to the idea that the Zinc-deprived animals exhibited more anxiety symptomatic behaviors than the Zinc-adequate animals as was reflected in the water maze.

Conclusions drawn on Corticosterone Plasma Measurement

Plasma drawn at baseline, one month, two months and three months was used as a check for the manipulation of corticosterone treatment. However, the measurement of corticosterone concentration unexpectantly resulted in the implanted animals having the highest concentration at baseline than at the three draws taken at one month, two months and three months after pellet implantation. Whereas the sham-surgery animals had the highest concentration at the end of the study draw (three months) than the other three draws including baseline.

This finding is in direct conflict with Bodnoff et al. (1995), who used the same dosage of corticosterone as this study. Bodnoff et al. assessed corticosterone concentrations at 2.5 months using radioimmunoassay and found levels in a range of 23-32 μg/dl However, it appears that Bodnoff et al. did not compare their levels against a control group, thus leading to the question of whether control animals put in the same situation would have similar or less concentrations of corticosterone. In addition, Dawson, Kontur and Mojan (1984) compared the concentrations found when using radioimmunoassay (as Bodnoff et al, 1995) and using HPLC (as was done in the present study). Dawson et al. found consistently lower concentrations of corticosterone using the HPLC procedure, but these values were highly correlated with the levels found using radioimmunoassay. Given the differences in measurement technique it is difficult to draw a comparison with the Bodnoff et al. study. This difference may also be due to adaptation or the development of tolerance of the animal to the stress of the blood draws and kill with the corticosterone animals already accustomed to a level of stress and thus

not reacting as much as the sham-surgery animals. The level of corticosterone, as a stress hormone is highly reactive to differences in level of stress.

The other explanation may lie in the animals physiological system accommodating the higher level of corticosterone produced by the implant and perhaps resulting in a shut down of the endogenous supply of corticosterone. This may have resulted in a lower overall corticosterone concentration. This explanation is supported by a study by Lin and Singer (1990), who also found lower levels in animals treated with a similar dose of implanted corticosterone but for ten days. The study's authors suggest that the level of corticosterone in the implanted animals represent only exogenous corticosterone exposure as the concentrations are similar to levels found in adrenalectomized animals with [illegible] implants.

Dietary treatment also resulted in an effect on corticosterone concentrations. The zinc-adequate animals had the highest corticosterone concentration at three months with this measurement greater than the Zinc-deprived animals. The Zinc-deprived animals had the highest level at baseline. If the theory of habituation or endogenous corticosterone shut-down holds true when examining dietary results, it appears that the Zinc-deprived animals had less of a response to the stress of the blood draws and the necropsy/perfusion than the Zinc-adequate animals.

Conclusions drawn on Weight and Food Consumption

The corticosterone-implanted animals were found to have consumed more overall food, which may have resulted in a higher body weight throughout the study as well as a higher body weight at the end of the study along with a heavier brain and liver than the sham-surgery animals. Some studies have shown increases in body weight and fat with

high levels of stress (i.e. Marniemi, Kronholm, Aunola, Toikka, Mattlar, Koskenvuo & Ronnemaa, 2002) would concur with this study. However, this increase in weight is in direct conflict with Sapolsky (1985) who found a reduction in body weight and a relatively large mortality rate. Sapolsky used Fisher-344 rats, which may have a different level of susceptibility to corticosterone than the Sprague-Dawley animals used in this study.

The lack of findings of weight differences between the dietary groups was unexpected. Previous research (i.e. Gordon, 1984) has shown that rats on a zinc-deprived diet lose weight and so traditionally, researchers have included a pair-fed group in order to control for the potential effects of weight loss on behavioral measures. The pair-fed group would receive the same amount of food as a matched zinc-deprived rat but the food would have an adequate amount of vitamins and minerals. This study also included a pair-fed group due to this rationale. However, it was found approximately two-thirds of the way through the study that the pair-fed group was unnecessary, as there was no difference in weight or food consumption between the Zinc-deprived and Zinc-adequate animals.

Conclusions drawn on Complete Blood Count

The red-blood-cell parameters reflect the general health and ability of the blood to carry oxygen and excrete wastes. There was a greater red blood cell count for the zinc-adequate animals as compared to the Zinc-deprived animals with the Zinc-deprived animals having a greater mean cell volume and mean cell hemoglobin than the Zinc-adequate animals. This may be indicative of a macrocytic condition in the Zinc-deprived animals as they have less red blood cells and more volume leading to the conclusion that

their red blood cells are greater in size. The mean cell hemoglobin was also found to be correlated with the corticosterone plasma measurement at one month with higher mean cell hemoglobin associated with greater corticosterone concentration. Latency of the water maze task during session two was found to be correlated with hematocrit. Latencies increased with increases in hematocrit.

The mean platelet volume resulted in a greater volume in the sham-surgery and zinc adequate animals than the sham surgery/Zinc-deprived animals and the corticosterone-implant, Zinc-adequate animals, showing the possibility of both diet and corticosterone resulting in reduced mean platelet volume. Also, all of the groups had a smaller platelet volume than the normal mean. A reduction in platelet volume may result in problems with clotting. Total platelet volume was found to be correlated with the total activity measurement on short-term activity with a higher total platelet volume associated with a greater total activity.

The white-blood-cell count and the differential of the different types of white blood cells is indicative of general immune functioning. The overall white blood cell count of the sham-surgery, Zinc-adequate animals was higher than the sham-surgery/Zinc-deprived animals and the corticosterone/Zinc-adequate animals.

The sham surgery animals had more lymphocytes than the corticosterone group. More lymphocytes were found in the sham-surgery/zinc-adequate animals than the sham surgery/Zinc-deprived animals and these animals had more lymphocytes than either corticosterone group (Zinc-adequate and Zinc-deprived). This demonstrated a relatively strong relationship between the corticosterone treatment and decreased lymphocyte percentage. However, all of the groups other than the sham-surgery, Zinc-adequate group

had less percentage of lymphocytes than the normal mean. Lymphocytes produce antibodies against foreign materials such as viruses and bacteria.

The corticosterone group had a greater neutrophil percentage than the sham-surgery group. The sham-surgery/Zinc-deprived animals had a greater neutrophil percentage than the sham-surgery/Zinc-adequate animals. The corticosterone-implanted, Zinc-adequate animals had a greater neutrophil percentage than the sham surgery, Zinc-adequate and Zinc-deprived animals. Neutrophils are used to combat bacteria, which may lead to the conclusion that the implanted animals had some sort of bacteria they were fighting with, perhaps as a result of the implant. The percentage of neutrophils was also correlated with the number of errors during session two of the water maze task with a greater percentage of neutrophils associated with a greater number of errors.

For eosinophil percentage the sham surgery/zinc-adequate animals had a greater percentage than the sham-surgery /Zinc-deprived animals and both groups of corticosterone treated animals. This demonstrates that the eosinophil percentage is reduced by zinc-deficiency and corticosterone treatment. Although all of the groups were within expected range of eosinophil percentage when comparing with the normal mean. Eosinophils are used to combat histamines or allergic reactions. The eosinophil percentage was also found to be strongly correlated with the zinc concentration of the thalamus with increases in eosinophil percentage associated with increases in Zinc concentration.

Limitations and Future Research Directions

Several limitations were found this study. The greatest was the difficulty with the pellet implantation. The experimenter chose to use two, 200mg corticosterone pellets

purchased from Innovative Research (Sarasota, Florida). These pellets were very large and inserting them was not as simple as the company claimed. Innovative products claimed that the pellets could be inserted through a small slit in the upper back of the animal with no stitches required. The pellets were easy to insert. However, the incision did require staples to enclose the large pellets. There were no problems associated with the healing of the original incision site, instead problems with the pellets later erupting through the skin of the animal was the primary problem. This usually occurred through skin that had grown necrotic, leading to the theory that the pellets were causing a local infection, which affected the health of the animal's skin. Once a pellet erupted through the skin, the pellet was cleaned as well as possible, using no solvents as this would have dissolved the pellet and it was inse[illegible] the animal. This experimenter believes the overall problem was with infection introduced with the pellet implantation or developing shortly after implantation. In future studies, an antibiotic powder or some other treatment should be used to prevent infection around the pellet. In addition, a smaller size pellet may not place so much stress on the skin and the surrounding tissue and may not produce as many concerns. Although, pellet re-implantation was a large problem in the study, and overall the animals implanted with the corticosterone pellets did have a decrease in plasma corticosterone concentration, it did not appear to affect the plasma levels of corticosterone as no difference was found between those animals with implant problems and those whom had no re-implantation surgeries. In addition, several behavioral and histological effects were found due to the corticosterone treatment.

A second limitation was the failure of the animals to learn the short-term memory task, the radial arm maze. Prior research has utilized similar training methods (i.e. Olton

& Samuelson, 1976) and have been successful in training the animals to enter each of the eight arms, retrieve a food pellet and then enter another arm without repeating an arm that has already been visited. The animals in this study visited very few arms during training and testing and consumed few pellets. Two possible explanations exist for these results. The first is that the animals were not fully trained and would have benefited from more training sessions. The animals received five days of training and it appears that those animals that performed the task, learned it fairly quickly in those five days. It is possible, that many other animals would have also learned the task if the number of training sessions were increased. The second possible explanation is that the animals were not properly motivated to complete the task. This lack in motivation may have occurred because the animals were not 85% body weight food-deprived as other studies have done. The reason this procedure was not used was because one of the independent variables in this study is diet, specifically zinc- adequate and Zinc-deprived diets, thus not allowing the animals to be food deprived. A solution to this problem has been to use a reinforcer within the maze that is more desirable than what the animals normally consume such as peanut butter or fruit loops. However, again because a trace mineral, zinc was being manipulated, using these reinforcers was not a possible solution. A compromise was originally proposed in which the animals were fasted beginning at 10:00 P.M. the night prior to testing and then received their food after testing was over the next day. This procedure was done in the current study with both the radial arm maze and the short-term activity task. However, this does not appear to be an effective solution to this motivation problem. It may prove difficult to have any behavioral task that relies on food

reinforcement used in a study with diet as a primary variable. This would explain the lack of studies in the trace mineral literature using food reinforcement behavioral tasks.

Another limitation was decreased statistical power due to a large amount of variability between animals and a small sample size. Many variables failed to reach significance due to this limitation. Future studies will need to include a greater number of animals in order to control for variability.

Several future studies are suggested. The first would be a more in-depth analysis of the effect of the corticosterone implants on plasma corticosterone concentrations than was done in the present study. After implanting four 100-mg pellets using an antibiotic substance to prevent infection, blood should be drawn on a weekly basis to better determine where in the time-line the concentration of corticosterone falls below the sham-surgery group and would serve to replicate this controversial finding. A comparison of environmental stressors with corticosterone administration to determine if there are differences in impact on memory and brain histology would also be of interest. The measurement of zinc concentration in the brain tissue of the animals along with the finding of no difference in weight and food consumption should also be replicated as these are also controversial findings and replication would strengthen these findings. Other animal studies of interest would include examining the interaction of corticosterone and zinc-deprivation on aging, startle response, EEG activity and an examination of potential gender differences. Human studies may examine the amount of daily or life stressors in the individual and compare with corticosterone concentrations, short-and long-term memory and dietary factors.

Moulton / Penland Zn 438 - Summary of Scheduled Activities

02/14/2001	Animals arrive and are started on AIN-93G with 12 ppm Zn.
	Animals are weighed, assigned a number and number is marked on tail.
06/23/2001	Animals are assigned to diet groups. ZnA (AIN-93M with 12 ppm Zn)
	ZnD (AIN-93M with 1 ppm Zn)
	ZnPF (AIN93-M with 12 ppm Zn restricted intake)

	Group A		Group B	
Date	**Week #**	**Activity**	**Week #**	**Activity**
6/25-6/30	Training	Subset 1: Radial Arm Maze Training Subset 2: Water Maze Training		
7/2 - 7/7	Training	**Holiday 7/4** Subset 1: Water Maze Training Subset 2: Radial Arm Maze Training		
7/9 - 7-14	0	Begin Diet Treatment, take baseline blood draw and insert glucocorticoid pellets. Animals fasted by 4:00 P.M. day before. Animal weights (7/11)	Training	Subset 1: Radial Arm Maze Training Subset 2: Water Maze Training
7/16 - 7/21	1	Animal Weights (7/18)	Training	Subset 1: Water Maze Training Subset 2: Radial Arm Maze Training
7/23- 7/28	2	22-hour Activity Session 1 Subset 1: Habituation (7/23), testing (7/24-7/25) Subset 2: Habituation (7/26), testing (7/27-7/28) Animal weights (7/25)	0	Begin Diet Treatment, take baseline blood draw and insert glucocorticoid pellets. Animals fasted by 4:00 P.M. day before. Animal weights (7/25)
7/30 - 8/4	3	Radial Arm Maze Session One: Rats fasted by 4:00 P.M. Animal weights (8/1)	1	Animal weights (8/1)
8/6 - 8/11	4	Blood draws. Animals fasted at 4:00 P.M. day before. Animal weights (8/8)	2	22-hour Activity Session 1 Subset 1: Habituation (8/6), testing (8/7-8/8) Subset 2: Habituation (8/9), testing (8/10 - 8/11). Animal weights (8/8)
8/13 - 8/18	5	Water Maze Session One: Path B Animal Weights (8/15)	3	Radial Arm Maze Session One: Rats fasted by 4:00 P.M. day prior to testing day. Animal weights (8/15)
8/20 - 8/25	6	Animal Weights (8/22)	4	Blood draws. Animals fasted by 4:00 P.M. day before. Animal weights (8/22)
8/27 - 9/1	7	Animal Weights (8/29)	5	Water Maze Session One: Path B. Animal weights (8/29)
9/3 - 9/8	8	**Holiday 9/3** Blood draws. Animals fasted by 4:00 day prior. Animal weights (9/5)	6	Animal weights (9/5).
9/10 - 9/15	9	TruScan Activity Testing: Rats fasted by 4:00 day prior to testing. Animal weights (9/12)	7	Animal weights (9/12)
9/17- 9/22	10	22-Hour Activity Session 2 Subset 1: Habituation (9/17), testing (9/18 - 9/19) Subset 2: Habituation (9/20), testing (9/21-9/22). Animal weights (9/19)	8	Blood draws. Animals fasted by 4:00 P.M. day before. Animal weights (9/19).
9/24 - 9/29	11	Radial Arm Maze Session Two: Rats fasted by 4:00 P.M. day prior to testing. Animal weights (9/26)	9	TruScan Activity Testing: Rats fasted by 4:00 prior to test day. Animal weights (9/26).
10/1 - 10-6	12	Water Maze Session Two: Path A. Animal weights (10/3)	10	22-Hour Activity Session 2 Subset 1: Habituation (10/1), testing (10/2-10/3) Subset 2: Habituation (10/4), testing (10/5-10/6). Animal weights (10/3)
10/8 - 10/13	13	Animals fasted by 4:00 P.M. day prior to necropsy. Necropsy/ Perfusion Blood draws done prior to perfusion.	11	Radial Arm Maze Session Two: Rats fasted by 4:00 P.M. day prior to testing. Animal weights (10/10)
10/15 - 10-21			12	Water Maze Session Two: Path A. Animal weights (10/17)
10/22 - 10/27			13	Animals fasted by 4:00 P.M. day prior to necropsy. Necropsy/ Perfusion Blood draws done prior to perfusion.

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