

# THE EFFECTS OF SEVERE ZINC DEFICIENCY ON THE SPATIAL WORKING MEMORY OF YOUNG AND ADULT FEMALE RATS

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## Abstract

A severe zinc deficiency was induced in 33 day old and 4.5 month old female rats to study the effects of zinc loss on spatial working memory at different developmental stages. A zinc deficient group fed a diet deficient in zinc (1 ppm) and a control pair fed group fed a standard rat chow diet were tested for spatial working memory abilities with an elevated T-maze. Results yielded a main effect for treatment, suggesting that both young and older zinc deficient rats performed worse on the T-maze than their pair-fed controls. The main effect was qualified with a treatment by age interaction, indicating that the spatial working memory of the younger zinc deficient rats were more severely affected by zinc loss than the older zinc deficient group. The response latency for the zinc deficient groups was significantly slower than the pair-fed groups. In addition, no significant results were found for the activity wheel tasks, suggesting that physical complications such as lethargy often associated with zinc loss did not affect the subjects' performance on the T-maze. The findings from this study suggest that zinc deficiency does have negative effects on spatial working memory. Deficiency at a young age more severely affects spatial working memory as compared to deficiency at an older age. The age of onset of a nutritional deficiency could determine its severity.

## Introduction

Zinc has been shown to be an essential element for human development. The most susceptible time for dietary zinc deficiency is during rapid growth periods such as pregnancy, infancy, and adolescence (Golub, Keen, Gershwin, & Hendrickx, 1995). Zinc deficiency at these ages can lead to temporary or permanent developmental complications. Although these time periods are the most vulnerable to zinc deficiency, problems can develop at any age since the main cause for zinc loss is the consumption of diets that are low in highly bioavailable zinc; therefore, this is a great concern not only for malnourished populations, but for all individuals with a poor quality diet, making it a public health problem.

Zinc is essential for brain maturation and function (Sanstead, Penland, Alcock, Dayal, Chen, Li, Zhao, & Yang, 1998). Zinc has been associated with several brain functions including DNA and protein synthesis at critical periods of brain growth, neurotransmitter function, hormone/growth factor transport and receptor binding. Furthermore, zinc has been found to be concentrated in the hippocampus, which is responsible for neuronal activity and memory formation (Wasantwisut, 1997). In the brain, zinc is found primarily in the hippocampus and its presence is nearly ten fold greater than any other trace metal in the region (Golub, Gershwin, & Vijayan, 1983; Halas, Eberhardt, Diers, & Sanstead, 1983). Normal synaptic transmission in the hippocampus depends on zinc in synaptic boutons and mossy fibers (Pfeiffer, & Braverman, 1982). This process is thought to be important for memory, suggesting a role of zinc in memory formation (Sanstead, 1985).

Since zinc has been associated with memory formation in the hippocampus, studies have been performed examining the cognitive effects of zinc deficiency in animals. Previous research has indicated that severe zinc deficiency in adult rats produces similar impairments as those produced by hippocampal lesions, suggesting that a zinc deficiency affects hippocampal functioning (Golub et al., 1995; Halas, et al., 1983; Halas, Hunt, & Eberhardt, 1986). Although there have been conflicting results regarding the specific role of hippocampal zinc in memory, memory impairments have been widely associated with zinc deficits.

In order to examine the effects of zinc deficiency on memory, several animal experiments have been performed. In one experiment, memory development was examined in young male and female rats severely deprived of zinc from birth until their 22nd postnatal day. It was shown that the zinc deprived animals had a significant delay in long term memory development, measured by a tone-shock conditioning task, as compared to their pair-fed control and *ad lib* control groups (Halas, Heinrick, & Sandstead, 1979).

This discrepancy cannot be attributed to malnutrition alone because the control group ate the same quantity of standard diet as the experimental group ate the zinc-deficient diet, and the control animals did not display a delay in development of long-term memory (Halas et al., 1979).

In another experiment, memory impairment was measured in adult rats given a severe zinc deficiency during lactation. Using a radial arm maze, which is specifically designed to measure spatial working memory, it was shown that the female rats who were zinc deprived during lactation performed significantly worse than their pair-fed control counterparts (Halas et al., 1983). Because zinc deficiency causes a loss in appetite, it has been argued that the zinc deficient animals performed worse because they were generally malnourished; however, in this experiment the female control animals, who were just as malnourished as the experimental group due to pair feeding, performed just as well as the ad lib animals on the radial arm maze (Halas et al., 1983). Similarly, in another maze learning experiment, it was shown that rats who were severely zinc deprived during lactation and then tested at 44 days of age made more errors than the pair-fed control group (Golub et al., 1995).

In a follow-up to their previous experiment linking spatial working memory impairments to zinc deficiency, Halas et al. conducted a study examining young adult rats born to mildly zinc deficient dams. Three experiments were conducted to test the behavior of offspring of rat dams fed a mildly zinc deficient diet during pregnancy and lactation (Halas et al., 1986). Similar to the last, this study uses a 17 arm radial maze, baiting all 17 arms to test working memory ability in the female subjects. Offspring of the ad lib and pair-fed groups performed significantly better compared to the zinc deficient group, while there were no differences between the offspring of the ad lib and pair-fed groups. Results demonstrate that the zinc deficient rats suffered a permanent working memory impairment even when fed a diet containing normally adequate levels of zinc post-natally. Overall, this research suggests zinc deficiency may have a more severe impact on the brain than undernutrition alone, represented by the pair-fed group. The pair-fed rats did not suffer from any working memory impairments and only had mild learning deficits, illustrated by the slower rate of maze learning (Halas et al., 1986).

A developmental study was performed to assess the effects of zinc deficiency on the behavior of older rats and compare the results to observations of zinc-deprived young developing animals. The study examined overall behavior including food intake, body weight, and open field conduct in the older and younger rat groups. Results show that zinc deficiency in both age groups induced severe adverse physical and behavioral effects. However, the most critical finding to this study was that zinc deficiency was produced faster and was more permanent in younger animals as indicated by the deaths of three young deficient animals before testing (Gordon, Bond, Gordon, & Denny, 1982; Halas, et al., 1986). No aging zinc deficient rats died during the experiment despite the longer duration of the experiment (Gordon, et al., 1982).

Much of the research gathered about the effects of zinc deficiency on cognitive abilities comes from previous nutritional studies that measure the effects of malnutrition in clinical human patients. Our study is among the few to use animals for studying neuropsychological impairments by manipulating zinc deficiency and controlling for other confounds from malnutrition. Among the previous studies using rats, research has focused on zinc-deficient mothers and the effects of the nutritional deprivation in their weanlings. Past experiments have not compared the differences between cognitive impairments of young rats and the impairments that may be acquired at an older age. In addition, we examined the effects of a short-term severe zinc deprivation on working memory, whereas other studies have used a moderate zinc deficient diet for a longer time. Although this acute deprivation is not ethical for studying malnutrition in humans, creating a short-term severe zinc loss in an animal model will heighten the effects of the deficiency making the effects of zinc loss more apparent.

In the following experiment, we performed a developmental study that examined the effects of a severe zinc deprivation on the working memory of both young and older female rats. To investigate the effect of zinc loss on working memory, we designed an experiment using 10 young and 10 post-pubescent female rats. Half of each age group was assigned to the experimental group and the other half served as control subjects; the experimental group was fed a zinc deficient diet while the pair-fed control group was fed standard rat chow. Since zinc deficiency causes anorexia (Kawamoto, Castonguay, Keen, Stern, & Hurley, 1985), it was necessary to have a pair-fed control group, giving the same quantity of food to the control group as was eaten by the experimental group in the previous 24 hours (Halas et al., 1983). Pair-fed control groups are seen to be an essential component of an experiment using severe zinc deprivation (Golub et al., 1995). Both groups were tested on an elevated T-maze for working memory deficits. On the fifth week of the experiment, another variable was added to further differentiate between the experimental

and the control groups. Response latency, which was the time that it took for each rat to finish each choice run, was recorded. This was undertaken to see if any of the animals were hesitant in making their decision for the correct arm during their choice run, indicating that they may have working memory impairments. In addition, all rats were run through an activity wheel to assess their level of activity throughout the experiment.

After examining previous research, we hypothesize that zinc deficiency has harmful effects on working memory in female rats. Further, we hypothesize that the effects of zinc deprivation in young female rats are more severe than in adult female rats. We predict that neither zinc deficient group will perform as well on the elevated T-maze trials as the pair-fed controls. More specifically, the number of choice errors made by the zinc deficient animals in each series of maze choices will be more than the number of incorrect choices made by the control-pair fed rats. In addition, the response latency of the experimental group will be slower than that of the control animals. We further predict that the younger zinc deficient females will not perform as well than the older deficient females on their t-maze trials.

## Method

Ten female, 33 day and 10 female 4.5 month Sprague Dawley rats were maintained on a Purina Chow standard rat diet for 7 days. Five old and 5 young rats were randomly assigned as the experimental group and fed an egg based severely zinc deficient pellet diet containing 1 ppm zinc by analysis (Halas et al., 1979; Gordon et al., 1982; Kawamoto, et al, 1986). The remaining 5 rats in each group were classified as controls and fed the standard rat chow diet, which contained 70 ppm zinc by analysis. Rats were pair fed so that the daily intake for each control animal was equal to the mean number of grams consumed by the zinc deficient rats in the previous 24 hours.

The environment was made as metal free as possible. All rats were housed individually in stainless steel cages held on stainless steel racks. Distilled water was given in plastic bottles with stainless steel spouts at lib. The diet was placed on the bottom of the cage prior to each feeding period. The laboratory was kept on a 12 hour light/dark cycle. Body weight was recorded in 3 day intervals and food intake was recorded every other day for each animal. All intake data was corrected for any spillage, which was carefully collected and weighed.

## Apparatus

Testing equipment included a wooden elevated t-maze. The start arm measured 60 cm long and each choice arm was 40 cm long. All arms were 10 cm wide. A removable wooden block was used to restrict access to one choice arm. An additional blocker was used to contain the subject in the start box. An activity wheel was also used with a 60 cm circumference.

## Procedure

**Training Period.** All rats were handled for 20 minutes per day for 7 days prior to training. After the handling sessions, the rats were randomly assigned to either the ZD group or the pair-fed control group. Each animal received standard lab chow on a 12 hour pair-feeding schedule. During a 7 day period, all rats were individually trained on an elevated T-maze. Rats were initially acquainted with the T-maze and subsequently were trained to choose on each trial the opposite of the previous forced choice arm. The correct choice arm was baited with vanilla wafer cookies. The rats were given 3 trials per training session. All rats were acquainted with the activity wheel in a 5 minute training period.

**Testing Period.** After training, the pair-feeding schedule was continued with the experimental group receiving the zinc deficient diet and the controls receiving the standard diet for 42 days. Each rat was taken individually from the holding box and placed in the start box. For the information run, one choice arm was blocked off and the other choice arm had the reinforcer. For the choice run, access was permitted to both choice arms. The start door was opened, and then closed behind the rat. As soon as the rat entered a choice arm with all four legs, a block was placed behind the rat to confine it to that arm. If the choice was wrong, the rat was confined for 30 seconds. If the choice was correct, the rat ate its reinforcer and then was placed back into the holding box. Inter-trial intervals were approximately 4 minutes. Starting with the fifth week of the experiment, the reaction time of rats was recorded during their choice trial run in the T-maze testing periods.

The order of the trials for each pair was counterbalanced by alternating the correct choice arm to be on either the right or the left. Entering the wrong choice arm was counted as an error. After each testing period, the rat was returned to its home cage. The rat was given their food after all tests were performed. All rats were tested every 4 days for the duration of the experiment. After each rat finished its daily trial, the maze was wiped with a damp sponge and mild detergent.

For activity wheel testing, each rat was individually taken from a holding box and placed in the starting box attached to the activity wheel. The trap door was opened and the rat was allowed to enter the wheel. The rats were given a 1 minute acclimation period prior to a 3 minute testing time. The experimenter recorded the number of turns in each testing period. At the conclusion of the testing period, the trap door was opened and the rat received a reward in the starting box. The rat was then put back into the holding box. Each animal had one trial per testing day. Data was recorded for the number of turns the animal made in the wheel.

## Results

### T-maze

T-maze scores (number of correct choices) were subjected to a mixed model analysis of variance having two levels of age (young and old), two levels of treatment (zinc and zinc deficient), and seven levels of weeks tested. For an alpha level of  $p < .05$ , the effect of treatment was found to be significant. The main effect of treatment yielded an F ratio of  $F(2, 16) = 24.47$ ,  $p < .001$ . The strength of the relationship, as indicated by  $\eta^2$ , was .61. The mean for no zinc treatment group ( $M = 2.36$ ,  $SD = .05$ ) was significantly lower than the mean for the zinc adequate group ( $M = 2.74$ ,  $SD = .05$ ), indicating that the no zinc treatment group made significantly more errors than the zinc adequate group (see Figure 1 and Figure 2). No significant main effect was found for age,  $F(2, 16) = 1.77$ ,  $p = .202$ . No significant main effect was found for weeks of treatment,  $F(2, 6) = 1.99$ ,  $p = .075$ .

Mean Number of Correct Maze Choices Per Week

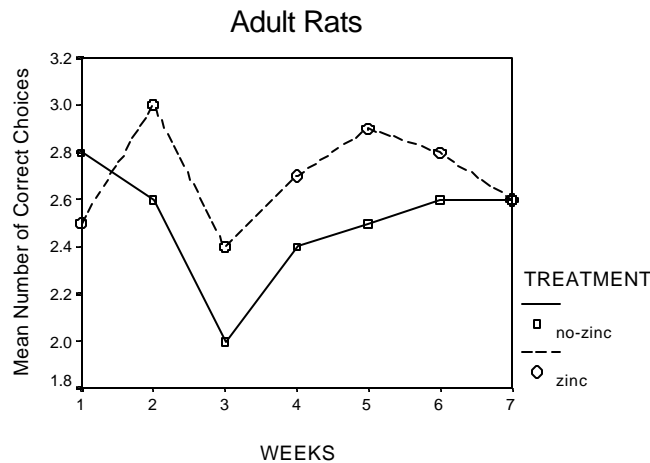


Figure 1. The mean number of correct maze choices that the adult female rats made over the seven weeks of the testing. The No-Zinc group made significantly more errors than the Zinc group.

### Mean Number of Correct Maze Choices Per Week

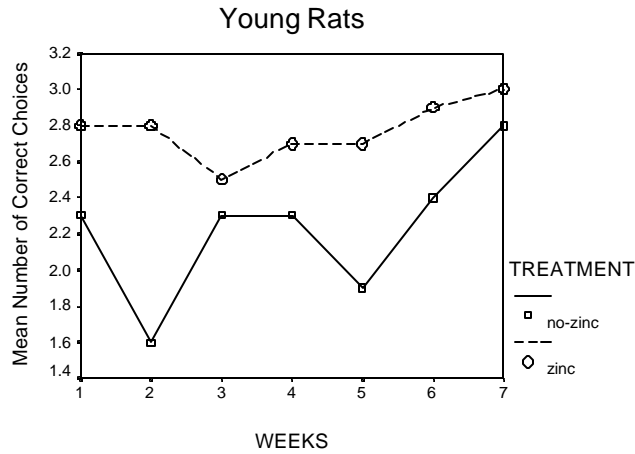


Figure 2. The mean number of correct maze choices that the young female rats made over the seven weeks of testing. The No-Zinc group made significantly more errors than the Zinc group.

Through analysis, an age by treatment interaction was revealed. The interaction effect,  $F(2, 16) = 5.21$ ,  $p = .036$ , was based on an overall alpha level of .05. The strength of the interaction effect, as indexed by  $\eta^2$ , was .25. The loss of zinc more severely affected the younger rats ( $2.77 - 2.23 = .54$ ) as compared to the older rats ( $2.70 - 2.50 = .20$ ) (see Figure 3). No treatment by week interaction was found,  $F(2, 6) = 1.53$ ,  $p = .18$ .

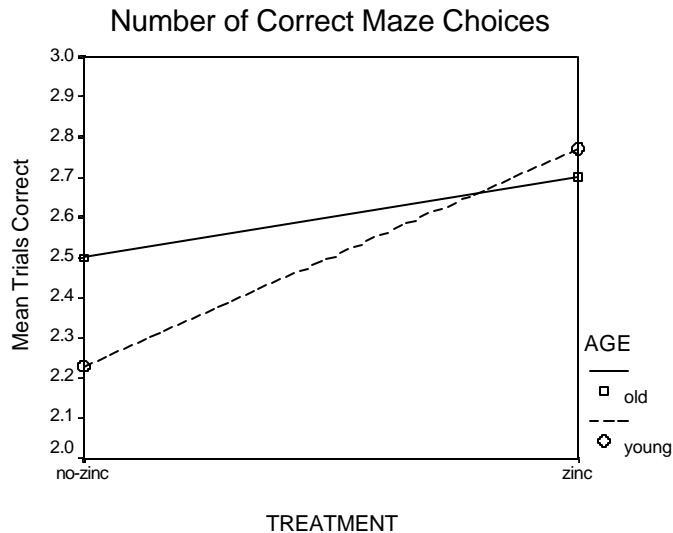


Figure 3. Treatment by Age interaction – The performance of the young rats was more severely affected by the zinc deficiency than the performance of the older rats.

An independent groups t-test was performed to compare the mean reaction time between the zinc and zinc deficient group for each week. Starting with week 5, the mean reaction time for the zinc adequate group ( $M = 1.91$ ,  $SD = .843$ ) was significantly lower than the zinc deficient group ( $M = 6.32$ ,  $SD = 4.16$ ),  $t(18) = 3.29$ ,  $p < .05$ . The strength of the relationship, as indexed by  $\eta^2$ , was .38. For week 6, the mean reaction time for the zinc adequate group ( $M = 2.54$ ,  $SD = .68$ ) was significantly lower than the zinc deficient group ( $M = 5.98$ ,  $SD = 3.36$ ),  $t(18) = 3.17$ ,  $p < .05$ . The strength of the relationship, as indexed by  $\eta^2$ , was .36. Week 7 produced the mean reaction time for the zinc adequate group ( $M = 1.98$ ,  $SD = .48$ ) was significantly lower than the zinc deficient group ( $M = 4.86$ ,  $SD = 1.96$ ),  $t(18) = 4.50$ ,  $p < .05$ . The strength of the relationship, as indexed by  $\eta^2$ , was .53. Overall, the reaction time of the zinc deficient group was significantly lower than the zinc adequate group.

## Activity Wheel

A one-way repeated measures ANOVA was performed to see if there were differences between the activity level of the zinc deficient groups and the control groups. No significant difference for the number of turns was found between the groups,  $F(1, 16) = .23$ ,  $p = .64$ , suggesting there was no difference of activity levels among the groups.

## Weight

A one-way repeated measures ANOVA was performed to compare differences of weight among zinc deficient and control groups. The mean weight for the zinc deficient group ( $M = 221.18$ ,  $SD = 3.16$ ) was not significantly different from the zinc adequate group ( $M = 216.43$ ,  $SD = 3.16$ ),  $F(1, 16) = 1.13$ ,  $p = .30$ . No interaction between the zinc deficient groups and control groups for either age was found,  $F(1, 16) = .045$ ,  $p = .83$ . The mean weight for the older zinc deficient group ( $M = 265.11$ ,  $SD = 4.46$ ) was not significantly different than the mean weight for the older zinc adequate group ( $M = 259.42$ ,  $SD = 4.46$ ) (see Figure 4). Similarly, the mean weight for the young zinc deficient group ( $M = 177.24$ ,  $SD = 4.46$ ) was not significantly different from the mean weight of the younger zinc adequate group ( $M = 173.44$ ,  $SD = 4.46$ ) (see Figure 5).

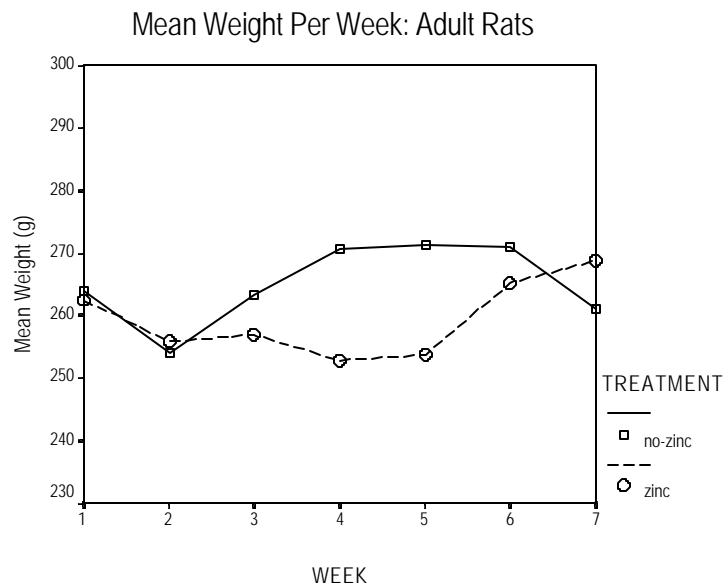


Figure 4. The mean weight of the adult rats over the seven weeks of testing. There was no significant difference between the older No-Zinc rats and the older Zinc rats.

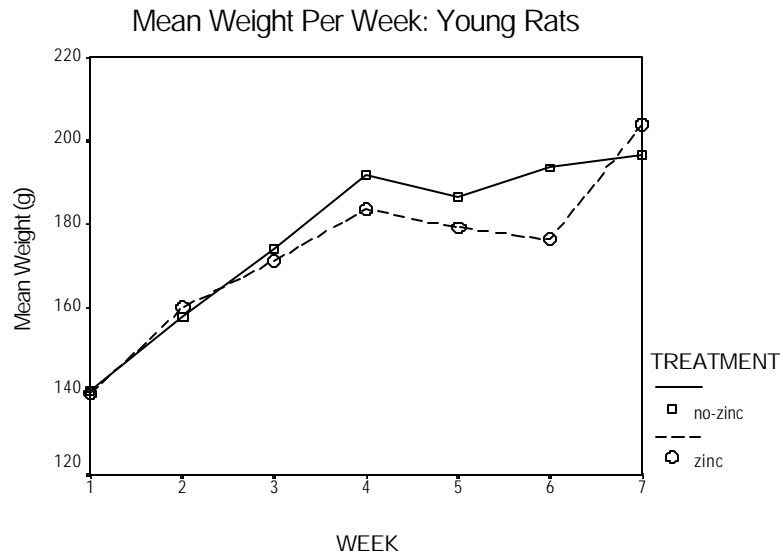


Figure 5. The mean weight of the young rats over the seven weeks of testing. There was significant difference between the young No-Zinc rats and the young Zinc rats.

## Discussion

The results from this experiment support our hypothesis that a lack of dietary zinc has detrimental effects on spatial working memory. As predicted, the zinc deficient animals performed significantly worse than their pair fed controls in the elevated T-maze. The animals suffering from zinc loss had fewer correct choices and slower reaction times in the T-maze than the control groups, suggesting that the zinc deficiency did cause working memory impairments across both age groups. In addition, the younger zinc deficient rats made fewer correct choices in the T-maze than the older zinc deficient group. These findings imply that the spatial working memory of the young rats was more severely affected by the zinc deficiency than the spatial working memory of the older rats.

To quantify further the differences between the two groups, response latency was introduced into the experiment during the fifth week. The zinc deficient groups were observed to be more hesitant than the control groups during their choice run. Results demonstrated that the zinc deficient rats vacillated significantly more before picking a choice arm than the control groups. The differing response times are likely attributed to working memory impairments. The differences in response latency were not due to a change in activity level because both experimental and control groups performed at a comparable level on the activity wheel measure.

The results from the working memory task were more reliable because the mean weights of the zinc deficient and control animals remained consistent throughout the experiment. Since the zinc deficient animals did not have a significant difference in weight from the control animals, the memory impairments incurred by the zinc deprived animals can be attributed more to the lack of zinc as opposed to general malnutrition. These results suggest that the confound of malnutrition was well controlled. The consistency among weights between the age groups is not a typical pattern found when studying zinc deficiencies. Usually anorexic effects are found in these experiments so that the zinc deficient animals weigh significantly less than their pair-fed counterparts (Kawamoto, et al., 1986). However, when adult female rats are deprived of zinc, they usually do not exhibit anorexic effects until the fourth week of deprivation. Analysis of zinc plasma levels in these animals did reveal a zinc deficiency, suggesting that female rats do not display anorexia until there is a prolonged deprivation. The young female zinc deficient rats in this experiment showed similar weight gains as other young female zinc deprived animals from previous experiments (Kawamoto, et al., 1986).

The results from the activity wheel also demonstrated that the spatial working impairments were due to a zinc deficiency and not general malnutrition. Activity wheel measures from both the zinc deficient and zinc adequate groups remained consistent throughout the experiment. This indicates that the response latencies of the zinc deficient rats were a result of zinc loss rather than general nutritional deficits. The lack

of zinc could have negatively affected their spatial memory processing, thereby slowing their decision making ability.

A developmental growth impairment seemed to have occurred across all groups. The weights of both the zinc deficient group and the pair fed controls stayed the same throughout the experiment. Although the pair feeding method controls for general malnutrition that may occur with anorexia, it also limits the amount of food intake of the control rats. Restricting the amount of food the controls can eat limits the amount of nutrients that their bodies can ingest. Therefore, the pair fed controls also suffer from nutritional deficiencies arising from malnutrition. An ad lib control group should be added to increase the validity of the method by providing a comparison to the performance of animals fed a sufficient quantity of nutritionally adequate food. This additional group does not undergo any manipulation and is therefore, a true control measure (Gordon, et al., 1982; Halas, et al., 1986).

The results from this experiment show that younger zinc deficient rats suffered more severe spatial working memory impairments than older zinc deficient rats. These findings propose that zinc is important for the normal cognitive development of young rats. A promising topic for future research would be to examine zinc's role in the hippocampus in relation to these cognitive impairments. Zinc is essential for the hippocampal development that occurs in rats postnatally during the first weeks of life. Research has indicated that zinc deficiency during this critical period for brain growth permanently affects brain growth (Pfeiffer, 1982). It is now necessary to examine the appropriate levels of zinc in the hippocampus and the cerebellar cortex necessary for normal neural development.

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## References

- Golub, M. S., Keen, C. L., Gershwin, M. E., & Hendrickx, A. G. (1995). Developmental zinc deficiency and behavior. *Journal of Nutrition*, 125, 2263s-2271s.
- Gordon, E. F., Bond, J. T., Gordon, R. C., & Deny, M. R. (1982). Zinc deficiency and behavior: a developmental perspective. *Physiology & Behavior*, 28, 893-897.
- Halas, E. S., Curtiss, H. D., & Eberhardt, M. J. (1986). Learning and memory disabilities in young adult rats from mildly zinc deficient dams. *Physiology & Behavior*, 37, 451-458.
- Halas, E. S., Eberhardt, M. J., Diers, M. A., & Sandstead, H. H. (1983). Learning and memory impairment in adult rats due to severe zinc deficiency during lactation. *Physiology & Behavior*, 30, 371-381.
- Halas, E. S., Heinrick, M. D., & Sandstead, H. H. (1979). Long term memory deficits in adult rats due to postnatal malnutrition. *Physiology & Behavior*, 22, 991-997.
- Kawamoto, J. C., Castonguay, T. W., Keen, C. L., Stern, J. S., & Hurley, L. S. (1986). Age, sex, and reproductive status alter the severity of anorexia in zinc deficient rats. *Physiology & Behavior*, 38, 485-493.
- Pfeiffer, C. C., & Bravenman, E. R. (1982). Zinc, the brain, and behavior. *Biological Psychiatry*, 17(4), 513-532.
- Sandstead, H. H. (1985). Zinc: essentiality for brain development and function. *Nutrition Reviews*, 43(5), 129-135.
- Sandstead, H. H., Penland, J. G., Alcock, N. W., Dayal, H. H., Chen, X. C., Li, J. S., Zhao, F., & Yang, J. J. (1998). Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. *American Journal of Clinical Nutrition*, 68, 470s-475s.
- Wasantwisut, E. (1997). Nutrition and development: other micronutrients' effect on growth and cognition. *Southeast Asian Journal of Tropical Medicine and Public Health*, 28, 78s-82s.