Cognitive effects of long-term enhanced dietary zinc consumption: Modulation by copper.

Railey, A.M.[1,*], Smith, L.N.[1], Micheli, T.L.[1], Morgan, K.[1], Fitzgerald, A.A.[1], VeeJay, M.[2], Flinn, J.M.[1], Wanschura, P.[1], Jones, B.F.[3], & Chrosniak, L.D.[1]

1. Dept. Psychology, George Mason University, Fairfax, VA, USA; 2. Thomas Jefferson H.S., Alexandria, VA, USA; 3.United States Geological Survey, Reston, VA, USA.

265.10

Introduction

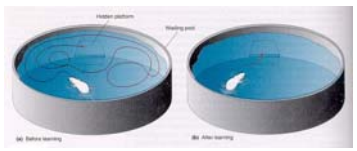
The use of zinc supplements has increased in recent years, particularly in the elderly, following the publication of an AREDS (2002) study reporting benefits of zinc supplementation with copper in the treatment of advanced macular degeneration. Most of the research involving zinc toxicity has focused on adverse effects at the cellular level, but there is little research involving the cognitive effects. However, chronic zinc consumption in drinking water has been shown to impair spatial memory in rats and wild-type mice (Flinn et al., 2005). The possibility exists that these memory impairments are a result of zinc's effect on copper. Elevated levels of zinc interfere with the absorption of copper through the intestinal wall. We sought to determine if adding a proportionate amount of copper to zinc-enhanced water could alleviate the cognitive deficits seen with chronic consumption of low levels of zinc. In order to examine this theory, we used cognitive tasks such as the Morris Water Maze (MWM) and a fear conditioning task.

Method

Male Sprague-Dawley rats were raised pre- and post-natally on zinc carbonate (10ppm $ZnCO_3$) (n=13), zinc carbonate plus copper chloride (10ppm $ZnCO_3$ + 0.2ppm $CuCl_2$) (n=12), or lab (tap) water (n=12).

Morris Water Maze

Task 1 assessed working and spatial reference memory in a Morris water maze (MWM) at 9 months of age. The MWM was a 6-ft diameter pool filled with water to 1cm above the top of a small escape platform hidden in one of the pool quadrants.



Atlantis Platform (spatial reference memory) involved 8 days of testing, with a stationary platform. Rats received three 60-second trials (A-C) per day for eight days. The platform was made unavailable every 6th trial (probe trials). Moving Platform (working memory) involved 4 days of testing, with the platform located in a new quadrant each day. Rats received four 60-second trials (A-D)/day.

Fear Conditioning

On the first day of testing rats spent 3 minutes habituating to the fear conditioning chamber. At 180 seconds, the CS was presented (85dB tone) for 20 seconds and co-terminated with a 2-second foot shock (1.0mA). The rats had two additional tone/shock pairings, exactly as described above, at the end of minutes 4 and 5, after which the animal remained in the chamber for 1 minute and was then returned to the home-cage.

On alternate testing days 2, 4, 6, 8, 10, 12, and 14, the animal was reintroduced to the testing chamber without the tone or shock, to assess the animals' memory of the contextual environment and then their extinction to it. On intervening days 3, 5, 7, 9, 11, 13 and 15 of testing, the chamber was altered with various shapes, lights, and floor texture to mask the training environment. The animal was placed in this altered chamber and received the same 20-second tone at the end of minutes 3, 4, and 5 as on the training day. This measured the animals' memory of the cue (CS), and their ability to extinguish the fear to the CS.

Results

Atlantis Platform

Escape latency: There was a difference in latency between water treatment groups on days 1-8, which approached significance (p=.067) (see Figure 1). An analysis of days 2-5 found a main effect of water type (F(2,33)=3.33, p<.05), post hoc tests showed this was due to the difference between zinc-treated animals and controls (see Figure 2).

Probe (C) Trials: A main effect of water type (F(2,33) = 4.47, p<.05), revealed that controls spent significantly more time in the target quadrant than zinc-treated animals on days 4,6, & 8 (see Figure 3). The zinc/copper-treated animals also spent more time in the target quadrant than zinc-treated animals, but did not differ significantly from either the zinc or the lab water group.

Fear Conditioning

Results were measured as percent freezing during a 60-second time period. An ANOVA demonstrated there was an overall significance for cued conditioning (F(2,33)=3.97, p=.029) for all 7 days of testing. Tukey HSD post-hoc analyses revealed a significant difference between the zinc and lab water groups within the first 3 minutes prior to the tone (p=.03) and the last 3 minutes (p=.039). An analysis of contextual results indicated no significance between the groups (F(2, 33)=2.72, p=.081) over all 7 days of testing.

Figure 1. Latency on Days 1-8 of the MWM, A&B trials.

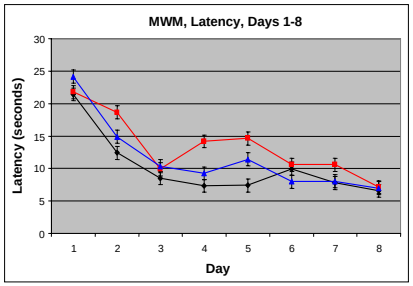


Figure 2. Latency on Days 2-5 of the MWM, A&B trials.

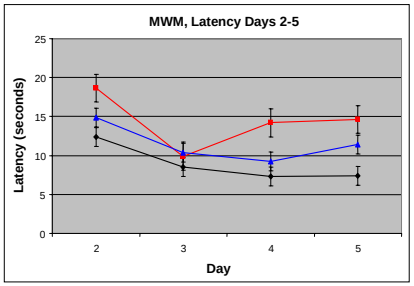


Figure 3. Percentage of time spent in the correct quadrant on probe trials, days 4,6,& 8 of the MWM.

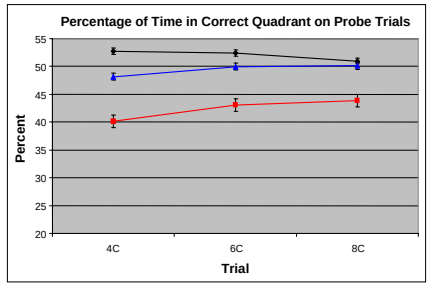


Figure 4. Percent freezing during Day 1 (training) of Fear Conditioning.

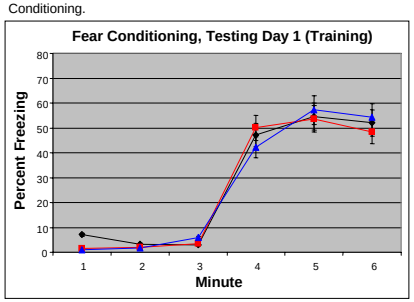
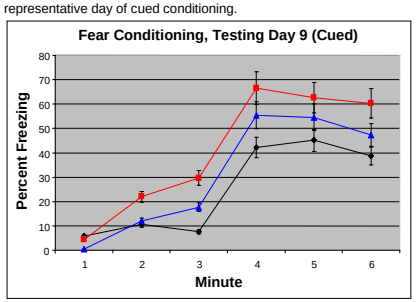
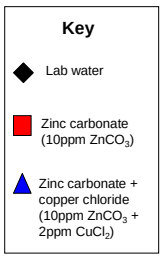


Figure 5. Percent freezing in response to the tone on a representative day of cued conditioning.





Conclusions

In this study, it appears that the addition of a small amount of supplementary copper reduces learning deficits associated with enhanced zinc exposure, suggesting that zinc related cognitive deficiencies may be due, at least in part, to copper deficiency.

The fear conditioning data suggest that, because auditory fear conditioning and extinction to the CS is amygdala and PFC dependent (Morgan & LeDoux, 1995), zinc may cause deficits in these brain regions as well as in the hippocampus. Impairment in the contextual component of the task may have been masked by high anxiety levels.

Acknowledgements and References

This study was supported by George Mason University and the United States Geological Survey.

Age-Related Eye Disease Study Research Group. (2002). AREDS report No. 7. *Journal of Nutrition*, *132*(4): 697-702.
Flinn, J.M., Hunter, D., Linkous, D.H., Lanzirotti, A., Smith, L.N., Brightwell, J., & Jones, B.F. (2005). Enhanced dietary zinc consumption in rats causes spatial memory deficits and increased zinc levels within the brain. *Physiology and Behavior, 83*(5): 793–803.
Morgan, M.A., & LeDoux, J.E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral Neuroscience*, *109*(4): 681–688.
MWM Diagram: Bear, M.F., Connors, B.W., & Paradiso, M.A. (1996). *Neuroscience: Exploring the Brain*. New York: Williams and Wilkins.