



Research report

Supplementation with zinc in rats enhances memory and reverses an age-dependent increase in plasma copper



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ABSTRACT

Zinc and copper are essential trace elements. Dyshomeostasis in these two metals has been observed in Alzheimer's disease, which causes profound cognitive impairment. Insulin therapy has been shown to enhance cognitive performance; however, recent data suggest that this effect may be at least in part due to the inclusion of zinc in the insulin formulation used. Zinc plays a key role in regulation of neuronal glutamate signaling, suggesting a possible link between zinc and memory processes. Consistent with this, zinc deficiency causes cognitive impairments in children. The effect of zinc supplementation on short- and long-term recognition memory, and on spatial working memory, was explored in young and adult male Sprague Dawley rats. After behavioral testing, hippocampal and plasma zinc and copper were measured. Age increased hippocampal zinc and copper, as well as plasma copper, and decreased plasma zinc. An interaction between age and treatment affecting plasma copper was also found, with zinc supplementation reversing elevated plasma copper concentration in adult rats. Zinc supplementation enhanced cognitive performance across tasks. These data support zinc as a plausible therapeutic intervention to ameliorate cognitive impairment in disorders characterized by alterations in zinc and copper, such as Alzheimer's disease.

1. Introduction

Zinc regulates many cellular processes, acting as a co-factor for more than 300 enzymes [1–8]. With only 2–4 g of zinc within the adult human body, concentration of zinc is tightly regulated [9]. Within the brain, zinc is concentrated in the limbic system [2,6,10], predominantly in the hippocampus and amygdala [2,11–13]; the hippocampus is the only brain area in which zinc increases markedly during development [14]. Zinc deficiency impairs cognitive and motor function in children [15,16] and damages the blood brain barrier [17], but less is known about the relationship between zinc and adult memory. Disturbances in zinc homeostasis have been associated with aging [18], Type II Diabetes (T2D) [9,19–22], and Alzheimer's disease (AD) [23–35], and this zinc dysregulation may contribute to the cognitive dysfunction seen in these disease states [36].

Zinc is co-secreted with glutamate. Zinc-containing glutamatergic neurons are dense in the mossy fibre layer of the hippocampus [1,5,37,38]: a zinc-deficient diet causes reduced neurogenesis and increased neuronal apoptosis within the hippocampus [39–42]. Vesicular zinc has recently been shown to be critical for hippocampal long-term potentiation (LTP) with both presynaptic and postsynaptic actions [43]. After secretion, zinc modulates postsynaptic excitability at NMDA

[44–47], dopamine [48], and GABA receptors [49,50]; zinc also modulates the Erk1/2 mitogen-activated-protein kinase (MAPK) pathway [51,52], brain-derived neurotrophic factor (BDNF) [10,53,54] and glucose metabolism [55,56]. An additional zinc-dependent enzyme recently identified as a potential modulator of hippocampal learning and memory is insulin-regulated aminopeptidase (IRAP) [57–60].

Since it was shown that zinc is a component of insulin crystals [61], a relationship between zinc and insulin signaling, a pathway known to impact learning and memory [62–64], has been proposed. More specifically, zinc causes tyrosine phosphorylation of the β subunit of the insulin receptor, increases phosphorylation of Akt serine residues and therefore activation of Akt, and induces an increase in glucose transport into cells via GLUT4 translocation [9,65–67].

Despite these several known effects of hippocampal zinc, zinc's role in adult cognitive impairment has not been extensively explored. Reduced zinc signaling produced by knock-out of the ZnT3 zinc transporter (responsible for packaging of vesicular zinc) produces cognitive impairment in 6 month-old mice, accompanied by marked glutamatergic dysfunction and a decrease in total dendritic spines per neuron [26] but this cognitive impairment was, interestingly, absent in young (6–10 week old) ZnT3 KO mice, suggesting that zinc regulation may be especially important in adult or aged brains.

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Recent research [68,69] suggests a possible link between zinc and a second essential micronutrient, copper, in the development and/or progression of Alzheimer's disease [30,33,70–76]. Copper can be toxic when in excess by contributing to oxidative stress [77]. Given this possible link, we measured both brain and plasma copper following zinc administration. Our overall hypothesis was that zinc supplementation might enhance hippocampally-mediated cognitive function, possibly via modulation of copper levels; and that this effect would be seen more clearly in adult rats than in young rats.

2. Material and methods

2.1. Participants

Male Sprague Dawley rats (N = 40; Charles River, Wilmington, MA), either 8-weeks-old (young group) or 24 weeks old (adult group), were pair-housed on a 12:12 h light:dark schedule with food and water available *ad libitum*. All procedures were approved by the University at Albany Institutional Animal Care and Use Committee. All rats were allowed to acclimate for at least one week prior to treatment. Animals were handled routinely from the time of their arrival by the experimenters to minimize any effects of handling stress on experimental measurements.

2.2. Zinc supplementation

Animals were randomly assigned to either regular drinking water or zinc-supplemented drinking water (75 mg/L elemental zinc) for two-weeks prior to behavioral testing. Animals remained on their assigned treatments during the behavioral testing schedule, totaling three weeks of treatment at the time of brain and blood collection.

2.3. Behavioral testing

2.3.1. Open field

Animals first completed an Open Field (OF) task. OF testing involved placing the animal into an open arena for 5 min. Time spent in the center zone and outer zone of the arena is recorded, with increased time in the center zone indicating lower anxiety. This task also served as a habituation trial for subsequent novel object recognition testing. The OF apparatus was cleaned with 70% ethanol in-between trials, ensuring no carry-over effects of scent influenced subsequent testing.

2.3.2. Novel object recognition

Thirty minutes after performing OF, animals performed Novel Object Recognition (NOR) to assess short- and long-term recognition memory. NOR testing involved placing the animals back into the open arena used in OF testing for three more 5-min trials. During the first trial, two identical objects were placed in the center zone and total time exploring both objects was recorded. Thirty minutes later, one of the identical objects was replaced with a novel object and the animals were returned to the behavioral apparatus. Time spent exploring the novel object as a percentage of total exploration time was calculated as 30-min novel object recognition. Twenty-four hours later, the animals were returned to the behavioral apparatus a second time, and a different novel object again replaced one of the identical objects seen during acquisition; a 24-h novel object recognition score was calculated in the same way. Higher scores are interpreted as indicating increased recognition memory. The objects used during NOR testing included a pair of water-filled 500 mL conical bottles, an opaque white teacup, and laboratory goggles. The OF apparatus and objects were cleaned with 70% ethanol in-between trials, ensuring no carry-over effects of scent influenced subsequent testing.

2.3.3. Spontaneous alternation

Forty-eight hours after completing NOR, animals were tested on the

4-arm Spontaneous Alternation (SA) spatial working memory task [62,78,79]. This task is known to be sensitive to alterations in hippocampal metabolism and insulin signaling [63,80–84]. Animals were placed in a 4-arm plus-shaped maze in which the animals utilized spatial cues to guide behavior. Alternation was calculated as the percentage successful alternations (entering each arm at least once in 5 attempts) of total possible alternations (number of arm entries minus four). Higher alternation scores indicate increased spatial working memory. The SA apparatus was cleaned with 70% ethanol in-between trials, ensuring no carry-over effects of scent influenced subsequent testing.

2.4. Atomic absorption analysis

Trunk blood and hippocampal tissue were collected at the time of sacrifice. Blood was immediately spun down and separated for plasma while hippocampi were removed and immediately homogenized and fixed in protease and phosphatase inhibitor buffers to prepare the tissue for atomic absorption (AA; Aurora Biomed Trace AI 1200) analyses. Plasma and homogenate were diluted with HPLC grade water to reduce background absorption. Zinc and copper concentrations were quantified using associated software relative to respective standard curves.

2.5. Statistical analyses

A 2×2 (age X treatment) ANOVA assessed the effects of age (8-weeks vs. 24-weeks) and treatment (control vs. zinc-supplemented), and the interaction of age and treatment, on open field, novel object recognition at 30-min and 24-h latency, and spontaneous alternation. A separate 2×2 (age X treatment) ANOVA assessed effects on plasma zinc, hippocampal zinc, plasma copper, and hippocampal copper. Significant main effects and interactions were further examined using post-hoc pairwise comparisons.

Statistical outliers were defined as falling more than two standard deviations from the mean for behavioral and biological analyses. If an animal was determined an outlier during the acquisition phase of NOR, subsequent trials (30-min and 24-h latency) were also removed from analyses (N = 5). If an animal was removed at any point during behavioral testing, it was also removed for biological analysis (N = 7; N = 1 removed from OF, N = 5 from NOR, N = 1 from SA). Samples that fell outside of the standard curve range during biological analysis and/or were more than two standard deviations from the mean were also removed (N = 6). One 24-week-old animal was removed from the study prior to treatment due to health problems, resulting in a total of N = 39 for behavioral and biological analyses prior to removal of statistical outliers and samples that fell outside of the standard curve. Group sizes for each analysis are as follows, Young Control: Open Field n = 10, NOR n = 8, SA n = 10, Plasma Zinc n = 8, Plasma Copper n = 8, Brain Zinc n = 8, Brain Copper n = 8; Young Zinc: Open Field n = 9, NOR n = 10, SA n = 10, Plasma Zinc n = 7, Plasma Copper n = 7, Brain Zinc n = 7, Brain Copper n = 7; Adult Control: Open Field n = 10, NOR n = 9, SA n = 10, Plasma Zinc n = 6, Plasma Copper n = 6, Brain Zinc n = 7, Brain Copper n = 7; Adult Zinc: Open Field n = 9, NOR n = 7, SA n = 8, Plasma Zinc n = 6, Plasma Copper n = 7, Brain Zinc n = 7, Brain Copper n = 7. Alpha was set at 0.05; data are shown as group means \pm SEM.

3. Results

3.1. Behavioral results

3.1.1. Open field

No effect of age or treatment on performance in the open field task was seen (all $p > 0.05$). Group mean times (\pm SEM) spent in the center zone were: Young Control 7.51 ± 2.03 s, Young Zinc 5.00 ± 1.82 s, Adult Control 7.29 ± 1.11 s, Adult Zinc

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