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Review

# Significance of $Zn^{2+}$ signaling in cognition: Insight from synaptic $Zn^{2+}$ dyshomeostasis

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ABSTRACT

Zinc is concentrated in the synaptic vesicles via zinc transporter-3 (ZnT3), released from glutamatergic (zincergic) neuron terminals, and serves as a signal factor ($Zn^{2+}$ signal) in the intracellular (cytosol) compartment as well as in the extracellular compartment. Synaptic $Zn^{2+}$ signaling is dynamically linked to neurotransmission via glutamate and is involved in synaptic plasticity such as long-term potentiation (LTP) and cognitive activity. Zinc concentration in the synaptic vesicles is correlated with ZnT3 protein expression and potentially decreased under chronic zinc deficiency. Synaptic vesicle serves as a large pool for $Zn^{2+}$ signaling and other organelles might also serve as a pool for $Zn^{2+}$ signaling. ZnT3KO mice and zinc-deficient animals, which lack or reduce $Zn^{2+}$ release into the extracellular space by action potentials, are able to recognize novel or displaced objects normally. However, the amount of $Zn^{2+}$ functioning as a signal factor increases along with brain development. Exogenous $Zn^{2+}$ lowers the threshold in hippocampal CA1 LTP induction in young rat. Furthermore, ZnT3KO mice lose advanced cognition such as contextual discrimination. It is likely that the optimal range of synaptic $Zn^{2+}$ signaling is involved in cognitive activity. On the basis of the findings on the relationship between dyshomeostasis of synaptic $Zn^{2+}$ and cognition, this paper summarizes the possible involvement of intracellular $Zn^{2+}$ signaling in cognitive ability.


**Contents**



## Introduction

Over 1000 proteins require zinc to carry out their functions in microorganisms, plants, and animals. Zinc powerfully influences cell division and differentiation [1–3]. Dietary zinc deficiency affects human health [4]. An estimated 17.3% of the world's population is at risk of inadequate zinc intake [5]. The marginal deficiency of zinc is not always correlated with the decrease in serum zinc in humans. On the other hand, dietary zinc deficiency easily decreases serum zinc levels in experimental animals [6]. Serum zinc is thought to be a major pool of zinc in the living body to transfer zinc to the tissues and organs. Zinc homeostasis in the living body is tightly controlled by both intestinal absorption and intestinal and renal excretions [7]. Zrt-Irt-like proteins (ZIP), which are responsible for the movement of zinc into the cytoplasm, and the zinc transporter (ZnT) family, which are responsible for the transport of zinc out of the cytoplasm, are involved in the absorption and excretions of zinc to maintain zinc homeostasis in the living body [8–10].

Zinc is critical for brain function as well as brain development [11]. In the brain, zinc is relatively concentrated in the hippocampus and amygdala [12]. Approximately 80% of the total brain zinc exists as zinc metalloproteins. The remaining part is histochemically reactive as revealed by Timm's sulfide-silver staining method

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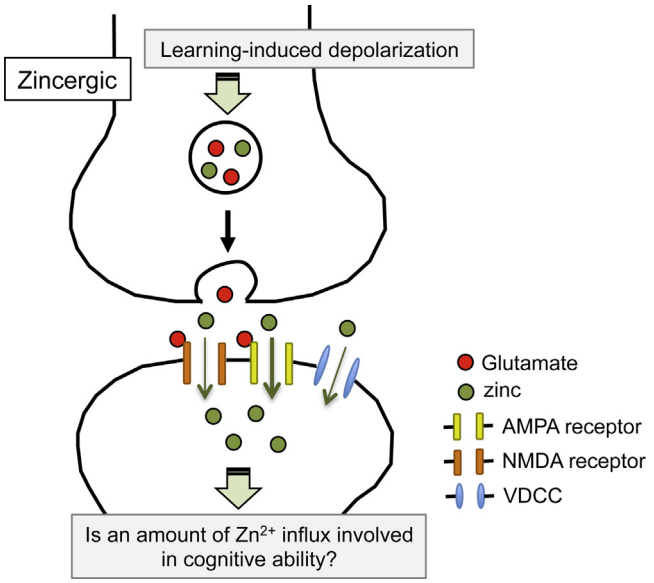


**Fig. 1.** Proposed role of synaptic $Zn^{2+}$ signaling in cognition. In zincergic synapses, the release of glutamate and $Zn^{2+}$ after LTP induction, which is increased in a depolarization frequency-dependent manner, induces $Zn^{2+}$ influx into the postsynaptic neurons. Calcium-permeable receptors and channels are involved in the rapid influx of $Zn^{2+}$ during synaptic excitation. The marginal reduction of synaptic $Zn^{2+}$ signaling may not significantly affect cognitive activity, while an optimal range of synaptic $Zn^{2+}$ signaling is potentially linked to memory retention and cognitive ability in advanced learning. The physiological significance of the degree of synaptic $Zn^{2+}$ signaling in cognitive activity remains to be clarified.

[13,14], The lack of ZnT3 protein, which is responsible for the movement of zinc from the cytoplasm into the synaptic vesicles results in 20% reduction of the total amount of brain zinc [15,16]. The zinc predominantly exists in the synaptic vesicle to serve as a $Zn^{2+}$ signal in the cytosolic compartment as well as the extracellular compartment and is also relatively concentrated in the hippocampus and amygdala [17,18].

$Zn^{2+}$ is released with glutamate from glutamatergic (zincergic) neuron terminals [19,20] and may modify glutamatergic excitation [22]. However, the range of optimal $Zn^{2+}$ levels in the extracellular compartment for synaptic function remains to be clarified. $Zn^{2+}$ released is immediately taken up into presynaptic and postsynaptic neurons through calcium-permeable channels such as calcium-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor, N-methyl-D-aspartate (NMDA) receptor and voltage-dependent calcium channels (VDCCs) during synaptic excitation (Fig. 1) [23–25]. The range of optimal $Zn^{2+}$ levels in the cytosolic compartment also remains to be clarified [26,27].

The spatiotemporal control of $Zn^{2+}$ signaling in both the extracellular and cytosolic compartments is important for synaptic function and the disruption of the control is involved in neurological diseases such as Alzheimer's disease [21,28–30]. On the basis of the findings on the relationship between dyshomeostasis of synaptic $Zn^{2+}$ and cognition, this paper summarizes the possible involvement of intracellular $Zn^{2+}$ signaling in cognitive ability.

## $Zn^{2+}$ homeostasis, zinc deficiency, and cognition

Extracellular zinc concentration in the adult brain is estimated to be less than 1 μM [31]. In vivo microdialysis experiments, on the other hand, zinc concentration in hippocampal perfusate is 100–200 nM [32,33]. If a recovery rate into the perfusate is around 10%, extracellular zinc concentration in the hippocampus is estimated to be more than 1 μM. In zincergic synapses, $Zn^{2+}$ concentration in the synaptic cleft may be higher than that in the brain's (extrasynaptic) extracellular fluid, because under hippocampal-slice-experiment conditions the regions where zincergic synapses exist are intensely stained by ZnAF-2, a membrane-impermeable zinc indicator [23]. The synaptic cleft is surrounded with the processes of astrocytes, which contribute to maintaining a steady concentration of zinc and neurotransmitters in the cleft. Interestingly, $Zn^{2+}$ level in the brain's extracellular fluid is estimated to be approximately 10 nM [34] and is higher than that in the plasma (<1 nM) [35]. On the basis of the synaptic $Zn^{2+}$ dynamics in the brain, it is possible that the ratio of $Zn^{2+}$ concentration to total zinc concentration in the extracellular compartment is relatively higher in the hippocampus where is enriched with zincergic synapses. There is some evidence that extracellular $Zn^{2+}$ might serve as a pool for zinc in the synaptic vesicle and might be involved in synaptic $Zn^{2+}$ homeostasis [36,37], although the chemical form of vesicular zinc is unknown; both vesicular zinc and extracellular zinc ($Zn^{2+}$) are responsive to dietary zinc deficiency. The increase in their levels along with brain maturation is suppressed by dietary zinc deficiency [36,37].

Zinc deficiency during pregnancy and during lactation has been shown to impair cognitive function and motor activity in offspring rats [38,39]. Young mice subjected to sever zinc deficiency for 5 weeks show spatial learning impairment [40]. Although the underlying mechanism of impaired learning and memory under zinc deficiency remains to be solved, it is possible that the increase in serum glucocorticoids is a factor to affect hippocampus-dependent learning and memory. Lupien et al. [41] reports that an elevation of basal cortisol may cause hippocampal damage and impair hippocampus-dependent learning and memory in humans. It has been reported that glucocorticoids may affect learning and memory processes by interacting with glutamatergic system [42,43]. In contrast, the induction of in vivo dentate gyrus LTP and object recognition memory are not affected in young rats after 4-week zinc deficiency, in spite of the significant increase in serum corticosterone, suggesting that abnormal corticosterone secretion does not affect cognitive function under zinc deficiency [37]. In this experiment, furthermore, extracellular $Zn^{2+}$ levels determined with ZnAF-2 are lower in zinc-deficient rats, in agreement with reduction of vesicular zinc levels determined by Timm's stain. Interestingly, dentate gyrus LTP and object recognition memory are affected in CQ (30 mg/kg)-administered rats [44], in which both extracellular and intracellular $Zn^{2+}$ levels are likely to be more markedly decreased in the hippocampus than in the zinc-deficient rats [45,46]. Therefore, the marginal reduction of extracellular $Zn^{2+}$ signaling, which is due to $Zn^{2+}$ release from zincergic neuron terminals, may not affect cognitive activity, i.e., object recognition memory, while a severe reduction, which is induced under unphysiological condition, affects it.

Basal $Zn^{2+}$ concentration is extremely low in the intracellular (cytosol) compartment (<1 nM) [47,48]. ZnT proteins such as ZnT1, ZnT3, and ZnT10, and Zrt-Irt-like proteins (ZIP) such as ZIP4 and ZIP6 are involved in the control of $Zn^{2+}$ levels in the cytosolic compartment, especially under static (basal) conditions [49,50]. Some of these transporters transport cytosolic $Zn^{2+}$ into a variety of subcellular organelles, including mitochondria, lysosomes, endosomes, and the Golgi apparatus, probably to maintain static $Zn^{2+}$ levels in the cytosolic compartment [51–53]. It has been reported that intracellular $Zn^{2+}$ signaling, which is linked to $Zn^{2+}$ release from zincergic neuron terminals, is involved in synaptic plasticity such as LTP; CA1 LTP is affected by the chelation of intracellular $Zn^{2+}$ with ZnAF-2DA, as well as the chelation of extracellular $Zn^{2+}$ with CaEDTA [54]. $Zn^{2+}$ is depolarization frequency-dependently released into the extracellular compartment in the hippocampus [55] and exogenous $Zn^{2+}$ lowers the threshold in hippocampal CA1 LTP induction in young rat [54]. It is possible that learning-induced $Zn^{2+}$ release from zincergic neuron terminals is involved in cognitive ability (Fig. 1). Intracellular $Zn^{2+}$ signaling is also required for mossy fiber LTP and $Zn^{2+}$ transactivates