# Zinc in Neurotransmission

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## Abstract

A subset of glutamatergic synapses in the central nervous system contains zinc; it is sequestered into the lumen of synaptic vesicles, where it colocalizes with glutamate. Extracellularly applied zinc is known to interact with various postsynaptic receptors and channels; however, the role of endogenous vesicular zinc is still an enigma. The aim of this review is to present the physiology of tonic and phasic zinc modulation of excitatory and inhibitory signals and to discuss the potential role of zinc in synaptic plasticity. Zinc homeostasis is known to be altered under pathological conditions. The importance of the careful investigation of the potential sources of zinc involved in physiological and pathological processes is highlighted.

## Contents



## INTRODUCTION

Under physiological conditions, free zinc concentration inside the cell is very tightly controlled. Zinc homeostasis is maintained by zinc-binding proteins, zinc sensors, and transporters localized on various intracellular elements. Zinc transporters (the ZnT family) can decrease intracellular free zinc concentration by transporting zinc ions from the cytoplasm to the lumen of organelles. In contrast, transporters of the Zrt-, Irt-like protein (ZIP) family increase intracellular zinc levels by transporting the ion from either the extracellular space or from intracellular organs into the cytoplasm. These regulatory mechanisms ensure that a vast majority of zinc inside the cell is tightly bound to various proteins, and only a small portion is chelatable or loosely bound. Zinc in this form can be found in forebrain glutamatergic neurons sequestered into synaptic vesicles (82) (**Figure 1*A*,*B***). Zinc-containing presynaptic terminals can be found in the olfactory bulb, neocortex, pyriform cortex, striatum, amygdala, and the hippocampus. The highest concentration of vesicular zinc is observed in hippocampal mossy fiber terminals (82). The role of vesicular zinc in synaptic transmission has been extensively studied in recent decades. On the basis of its localization, it was natural to assume that upon vesicle fusion, it is released to the synaptic cleft and acts as a neuromodulator. However, the emerging picture of how zinc influences neurotransmission seems to suggest a more complex interaction between neuronal signaling and zinc ions (53, 81).

**ZIP:** Zrt-, Irt-like protein

**ZnT3:** zinc transporter 3

## ZINC IN SYNAPTIC VESICLES

Zinc is transported into synaptic vesicles from the cytosol via a neuron-specific transporter, zinc transporter 3 (ZnT3) (78). Synaptic vesicles can be generated via two distinct pathways. Vesicles can be recycled directly from the plasma membrane via an adaptor protein 2 (AP2)-dependent pathway or via a longer route that involves intermediate endosomes. This latter pathway involves a different adaptor protein, AP3 (24). The fact that vesicles can recycle via two different pathways opens the possibility for the generation of molecularly diverse vesicle pools. PC12 cells were used to elegantly demonstrate that in synaptic-like microvesicles (SLMVs) pharmacological disruption of AP3-dependent SLMV biogenesis preferentially reduced ZnT3 targeting (88). These data are in good agreement with the observed phenotype of the AP3-deficient mocha mouse (49, 78). These animals show dramatically decreased levels of ZnT3, hence presynaptic terminals contain only minimal amounts of chelatable zinc. AP3-dependent mechanisms are also responsible for the selective targeting of a chloride channel, CIC-3, into the same vesicle pool (87). In addition, the tetanus neurotoxin-insensitive vesicle-associated membrane protein, which is expressed selectively in hippocampal mossy fibers under normal conditions, is absent from mocha mutant mice (90). Expression of these proteins on a subset of vesicles will generate a physiologically diverse vesicle pool in the presynaptic terminal.

Interaction between vesicular membrane proteins will determine the final vesicle content. ZnT3 and vesicular glutamate transporter 1 (VGlut1) are cotargeted to the same vesicle population, and they reciprocally influence glutamate and zinc uptake. Vesicular zinc uptake was increased by VGlut1 expression and ZnT3 increased glutamate uptake in a zinc-dependent fashion (86). In synaptic vesicle fractions from CIC-3 knockout (KO) animals, decreased VGlut1 content and vesicular glutamate uptake was observed (97). High luminal chloride concentrations are known to enhance glutamate loading by the facilitation of membrane-potential-driven uptake (89). However, since VGlut1 has also been shown to induce chloride conductance (5), and VGlut1 and not CIC-3 seems to be the major chloride permeation pathway in synaptic vesicles (89), interaction between these two vesicle membrane proteins remains enigmatic. Zinc staining is not observed uniformly in every vesicle (59, 82), and only a small fraction of the vesicles are labeled with Timm staining. Taken together, these data suggest that zinc-containing vesicles represent a subpopulation of the total vesicle pool with a unique composition of membrane proteins.

Synaptic activity is known to alter zinc content of the presynaptic terminal; neonatal whisker trimming leads to increased zinc staining in the centers of the deprived barrels in lamina IV (57). Although this change seemed to be permanent, whisker trimming in adult rats altered zinc levels temporarily in deprived barrel hollows. Zinc levels returned to normal values two to three weeks after whisker removal (8). In contrast, increased whisker activity led to selective decrease in zinc levels within layer 4 of the barrel hollow corresponding to the stimulated whisker (10). Although these studies demonstrate activity-dependent changes in presynaptic zinc levels, they cannot determine whether the observed changes were due to alteration in the number of zinc-positive terminals, vesicular zinc content, or the number of zinc-positive vesicles. In a recent study, however, change in the number of terminals containing zinc-positive vesicles was observed in the corresponding barrel cortex following whisker plucking (76). This result does not exclude the possibility that activity-dependent changes in zinc levels could also result from altered intravesicular zinc content or modified distribution of zinc-containing vesicles in the presynaptic terminals. It is currently unknown which mechanisms are responsible for activity-dependent changes in zinc levels and its functional consequences. Future experiments will determine whether the altered balance between AP2- and AP3-dependent endocytosis could contribute to the above-mentioned changes in synaptic zinc levels.

**VGluT1:** vesicular glutamate transporter 1

**KO:** knockout

**AMPA:** α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid

## EXCITATORY NEUROTRANSMISSION

### AMPA

Native AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate acid) receptors are mildly potentiated by a lower concentration of extracellularly applied zinc and inhibited when applied at a higher concentration (>500 μM) (64, 69). Similar results were obtained using recombinant receptors (29). In hippocampal slices, lower concentrations of zinc had no effect on synaptic AMPA responses (75). Whether zinc modulates AMPA responses is determined by the subunit composition, $Ca^{2+}$-permeability, and the splice variants of AMPA receptors. Zinc potentiates AMPA receptors containing the GluR3 subunit; this effect is observed over a narrow range of zinc concentration (~5 μM) (29). Dual modulation of AMPA receptors by zinc applied at different concentrations was observed in the flip but not in the flop variant receptors (92). Although zinc had dual effects on $Ca^{2+}$-permeable AMPA receptors, it did not affect signals transmitted by $Ca^{2+}$-impermeable AMPA receptors in the carp retina (100). The effect of zinc chelation on activity-dependent modulation of AMPA receptor–mediated signals has not been investigated. These data indicate that certain types of AMPA receptors could be influenced by extracellular zinc; however, it is unknown

whether they are modulated by endogenous zinc sequestered to synaptic vesicles.

**NMDA:** N-methyl-D-aspartic acid

**CaEDTA:** calcium disodium ethylenediamine tetraacetate

## Kainate

Recombinant GluR6/KA2 and GluR6 kainate receptors were inhibited by extracellular zinc applied in the μM range (35, 42). Native kainate receptors at hippocampal mossy fibers were inhibited by extracellularly applied zinc, and this effect was reversed by zinc chelators and could not be observed in *mocha* mutant mice. In these animals, zinc chelators could not further increase the kainate receptor–mediated fEPSP (excitatory postsynaptic potential) in the mossy fiber pathway. Proton-sensitivity of kainate signals was largely influenced by the receptor subunit composition, with KA2-containing receptors showing increased proton sensitivity (75). Taken together, these data indicate that native kainate receptors are regulated by endogenous zinc in a subunit-specific manner.

## NMDA

Selective inhibition of N-methyl-D-aspartic acid (NMDA) receptors by low micromolar concentration of zinc was first demonstrated in cultured hippocampal neurons (83, 108). Zinc acts in a voltage-independent fashion as an allosteric inhibitor to alter open channel probability (18, 19, 61, 69). The other mechanisms by which zinc can alter NMDA receptor function is a low-affinity voltage-dependent inhibition (15, 79, 109). Voltage-independent zinc sensitivity is largely influenced by the subunit composition of the receptor: NR1/NR2A shows the highest sensitivity (nM range), NR1/NR2B receptors have intermediate $IC_{50}$ values (1 μM), and NR1/NR2C exhibit the lowest sensitivity (15, 79, 84, 103). The sensitivity of NR2A-containing receptors is high enough to be tonically occupied by ambient zinc; in fact, this binding site can be studied only when zinc is properly buffered in the standard solutions (51, 79). Since zinc in the nM range can inhibit these receptors, it is conceivable that under physiological conditions, this binding site is tonically inhibited and is not able to sense activity-dependent changes in extracellular zinc levels. The high-affinity zinc-binding site has been mapped to the large NR2 amino-terminal domain (NTD) of the NR2A subunit (18, 32, 37, 66, 80). The NTD controls the subunit-specific gating of NMDA receptors and this sets the sensitivity to endogenous inhibitors such as zinc and protons (38) (**Figure 2*A***). NR2A-NTD binds zinc with high affinity; NTD of NR2B but not of NR2C or NR2D subunits forms low-affinity zinc-binding site (84). NMDA receptors incorporating different NR2 subunits can be modulated with widely different concentrations of extracellular zinc.

Although these experiments elegantly demonstrate that NMDA receptor function can be altered with extracellular application of zinc, surprisingly few experiments were dedicated to the study of the role of endogenous zinc. The most convincing data indicating that zinc modulates NMDA receptor–mediated synaptic responses in an activity-dependent manner showed differential regulation of low- and high-affinity binding sites (104) (**Figure 2*B***). Although the high-affinity, voltage-independent inhibition was unaltered by zinc chelation, the low-affinity voltage-dependent zinc block could be removed with calcium disodium ethylenediamine tetraacetate (CaEDTA). Using ionophoretic application of glutamate, the authors showed that mossy fiber activity contributes to the inhibition of NMDA receptors in the stratum lucidum, strongly suggesting that zinc is operating in a phasic fashion. In contrast, the high-affinity binding site is tonically occupied by zinc and therefore unable to alter NMDA function in an activity-dependent manner. Zinc chelation could effectively remove tonic inhibition from NMDA receptors at the recurrent mossy fiber synapses in pilocarpine-treated animals (74). This tonic inhibition seems to be pathway specific, as a similar effect was not observed at the perforant pathway or with the stimulation of the stratum radiatum in the CA3 area, or in the CA1 region, where NMDA responses at the Schaffer collaterals were not affected by CaEDTA application (46)

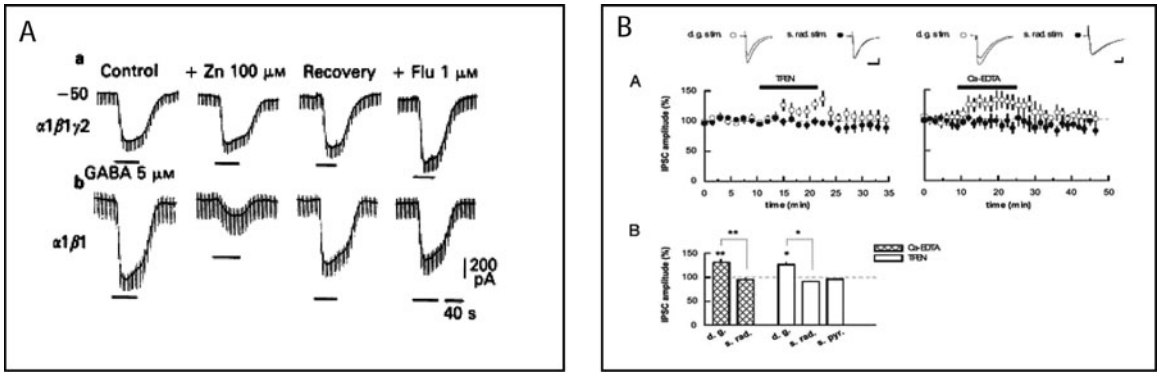


**Figure 3**

Zinc and GABAergic function. (*A*) Zinc inhibition is expressed in a subunit-specific manner; GABAA receptors containing the γ subunit were insensitive to extracellularly applied zinc. (From Reference 96, with permission.) (*B*) The effect of zinc chelation on inhibitory synaptic signals. N,N,N′N′-tetrakis(-)[2-pyridylmethyl]-ethylenediamine (TPEN) and calcium disodium ethylenediamine tetraacetate (CaEDTA) both increased IPSC amplitude possibly by removing the tonic zinc block from GABAA receptors. (From Reference 85, with permission.)



## INHIBITORY NEUROTRANSMISSION

Extracellularly applied zinc can influence the amplitude and kinetics of inhibitory postsynaptic currents (IPSCs) in a subunit-specific manner (28, 96) (**Figure 3*A***). The presence of the γ subunit rendered γ-aminobutyric acid $(GABA)_A$ receptors insensitive to extracellularly applied zinc, whereas receptors composed of αβ and αβδ were blocked by zinc in a noncompetitive and weakly voltage-dependent manner. The block observed in these recombinant receptors was comparable to the zinc block observed on GABAergic currents in neuronal cells (68, 94, 108). Zinc sensitivity of inhibitory signaling is developmentally regulated; embryonic neuronal GABAergic responses are antagonized by zinc, whereas adult responses are less sensitive (95, 96). This correlates well with the changes observed in expression pattern of various $GABA_A$ receptor subunits during development. In immature neurons, the expression level of α1, α4, and γ2 subunits was several-fold lower than in adult neurons (7). Differences in the subunit composition of synaptic and extrasynaptic GABA receptors are well documented. Although in synaptic specializations the αβγ isoform generally is expressed, GABA receptors containing the δ subunit have been found exclusively in the extrasynaptic somatic and dendritic membranes (77, 105). These studies indicate that in the adult network, phasic GABAergic transmission is not influenced by zinc. It is likely that extrasynaptic receptors are responsible for tonic GABAergic signaling (31); hence, zinc modulation of GABAergic receptors potentially could influence the tonic GABAergic signal. The origin of zinc that reaches the extrasynaptic sites is not known. In early postnatal slices, zinc chelation led to a decrease in the frequency of spontaneous large depolarizing potentials, while glutamate or GABA action on basic membrane properties were unaffected (111). Extracellular zinc chelation also increased IPSCs recorded from CA3 pyramidal cells with the stimulation of the dentate gyrus, while events evoked from the stratum radiatum were unaffected (**Figure 3*B***). However, responses to exogenously applied GABA could not be altered by the activation of mossy fibers (85). Because zinc chelators cannot differentiate between tonic and phasic zinc modulation, these results can be explained with the idea of a zinc veneer, which was proposed by Kay (50, 52, 53). In this view, presynaptic zinc would not be released upon stimulation but would stay loosely bound

**GABA:** γ-aminobutyric acid

**LTP:** long-term potentiation

to the presynaptic membrane when vesicles fuse with the plasma membrane. This idea is supported by the changes observed in the distribution of zinc staining upon increased synaptic activity at the ultrastructural level. Zinc staining is restricted to synaptic vesicles in control animals but appears at the presynaptic membrane surface in kainate-treated rats. Zinc staining in epileptic animals outlines the presynaptic membrane but does not appear in the synaptic cleft or on the postsynaptic membrane (59). Experimental tools that can properly differentiate between the physiological consequences of the removal of externalized loosely bound zinc and synaptically released zinc are not readily available.

An elegant and more specific alternative to zinc chelation is the genetic alteration of a specific zinc-binding site of a receptor. Elimination of the potentiating effect of zinc on α1-subunit-containing glycinergic currents led to a striking phenotype: Mice developed neuromotor deficiencies resembling human hyperekplexia (41). This study demonstrates that the presence of zinc is essential for normal glycinergic function. However, with this approach it is not possible to determine where the endogenous zinc originates. With the deletion of the zinc-binding site, both tonic and phasic modulation are compromised. Extracellular zinc application did not alter glycinergic signals in wild-type animals, and zinc-chelation depressed the IPSPs, suggesting that the zinc-binding site is persistently saturated, leaving no room for the detection of activity-dependent changes in zinc levels.

## SYNAPTIC PLASTICITY

Zinc concentration reaches the highest level in hippocampal mossy fiber terminals (**Figure 1*A***). The mechanism of long-term potentiation (LTP) is rather unusual at these synapses, as it is expressed primarily on the presynaptic site (113, but see 112). The high zinc content and the presynaptic form of LTP are unique features of this synapse; assuming that the two might be linked was an intriguing idea. Whether zinc is a key player in mossy fiber LTP was investigated by several laboratories that reached diametrically different conclusions. Zinc chelation or genetic ablation of ZnT3, rather than extracellular zinc application, should be used to determine the role of endogenous zinc because the concentration of released or externalized zinc has not been unequivocally determined as of yet. Mossy fiber inputs in in vitro slices are evoked with the stimulation of the dentate gyrus; however, due to the complex network wiring of this area of the hippocampus, extra care is needed for the proper isolation of these events. Stimulation of the dentate gyrus will lead to the activation of excitatory and inhibitory polysynaptic events yielding to a synaptic current, which is the result of these interactions instead of a simple monosynaptic input. Mossy fiber events need to be identified on the basis of their unique short-term plastic properties and with pharmacological tools that selectively suppress these events (48, 102). For these reasons, I focus only on publications that used zinc elimination and where mossy fiber inputs were properly identified. Although a lower concentration of CaEDTA had no effect on mossy fiber LTP, 10 mM CaEDTA was shown to block this form of synaptic plasticity (63). It was also suggested that actual translocation of zinc from the presynaptic site into the postsynaptic cell was a key factor in the mechanism of zinc action. However, it is not clear how postsynaptic zinc accumulation would interfere with synaptic plasticity in the presynaptic terminal. Moreover, higher concentrations of CaEDTA can interfere with intracellularly localized zinc; our data showed that CaEDTA at concentrations over 5 mM could effectively alter intracellular zinc pools (59). Using a lower concentration of CaEDTA, Vogt et al. (104) observed no change in mossy fiber LTP, and they also showed that *mocha* mice express normal mossy fiber LTP. These results convincingly demonstrated that neither zinc chelation nor genetic alteration of vesicular zinc can effectively interfere with mossy fiber LTP. However, two technical issues should be taken into consideration:

CaEDTA chelation might not be fast enough to capture zinc in the synaptic cleft (81), and mocha mice do not lack completely vesicular zinc (98). The apparent disconnect between postsynaptic zinc accumulation and the presynaptic form of LTP was addressed in an elegant publication by Huang et al. (43). The authors suggest that zinc in the postsynaptic cell regulates the Src family of protein tyrosine kinases and Src kinase activity leading to the activation of TrkB, and a retrograde signal to the presynaptic terminal would increase glutamate release. In this experiment, zinc chelation had a more robust effect on mossy fiber LTP than did the elimination of BDNF with genetic manipulations, indicating that zinc activation of TrkB receptors in the absence of BDNF is sufficient to induce LTP. In these experiments, zinc was chelated with a relatively high concentration of CaEDTA (7.5 mM). It would be interesting to see what is the minimum concentration of CaEDTA that is required to see this effect and whether in the absence of vesicular zinc or in the *mocha* mouse similar alterations of mossy fiber LTP could be observed. It is possible that in these animals LTP is unaffected, which would indicate that the observed chelator effect is due to the removal of tonically present zinc from a yet unidentified cellular element and not due to the blockage of zinc translocation. This picture is further complicated by the observation that endogenous BDNF action in mossy fibers is not sensitive to zinc chelation. This indicates that transactivation of TrkB receptors by zinc is independent of the endogenous action of BDNF activating TRPC3-mediated currents (62).

Zinc chelation could potentially influence other cellular mechanisms, as several metalloproteases have active zinc-binding sites. One such membrane protein is ADAM10, which binds zinc and is catalytically active (110); it has also been shown to be involved in the cleavage of ephrin (47). B-ephrin reverse signaling is critically important for mossy fiber LTP (4, 22).

LTP evoked at Schaffer collateral synapses was blocked by the application of CaEDTA even when applied and removed prior to high-frequency stimulation, whereas CaEDTA had no effect on NMDA EPSPs (46). This effect was attributed to the removal of tonic zinc inhibition from presumably extrasynaptic NMDA receptors. Unlike extracellularly applied zinc, zinc chelation will identify the role of endogenous zinc. However, its effectiveness alone does not necessarily indicate that endogenous zinc is released in an activity-dependent manner from the presynaptic terminal.

Outside the hippocampus, the role of zinc in synaptic plasticity was investigated in the amygdala, where LTP plays a role in fear conditioning (55). The authors found that zinc enables LTP at the cortico-thalamic synapse by depressing feedforward GABAergic inhibition. In the spinal cord, zinc chelation failed to alter long-term potentiation of C fiber–evoked potential (67).

## DIETARY ZINC AND CENTRAL NERVOUS SYSTEM FUNCTION

Zinc is an essential dietary trace element, and nutritional zinc deficiency has adverse effects on brain development. Severe dietary zinc restriction during early brain development was shown to cause malformations (44) and diminished performances in memory tests. Severe zinc deprivation during pregnancy has led to poorer performance of offspring in several behavioral tests, such as T-maze spatial discrimination and tone-shock conditioning (39). Zinc deficiency in adult animals has milder functional consequences: Poor performance in behavioral tests can be observed only following a prolonged period of severe zinc deprivation. Inferior learning was reported when one-month-old rats were fed a zinc-free diet for 48 days (12). Zinc restriction has been shown to influence short-term memory in rats only when the zinc-restricted diet is fed to animals less than 62 days of age (54). Similar behavioral effects were observed in prepubertal rhesus monkeys (40).

Zinc deprivation during embryonal development results in more severe functional outcomes, which could be attributed at least partly to the effect of zinc deficiency on neurogenesis and apoptosis. Lack of proper zinc uptake during the early postnatal period can delay the division and migration of cerebellar granule cells (30). Zinc deficiency can inhibit cell proliferation by cell cycle arrest at the $G_0/G_1$ phase (1). It also induces apoptosis by altering several pro-survival and proapoptotic pathways (1). A zinc-free diet fed to rats for six weeks significantly decreased the number of progenitor cells and immature neurons in the hippocampal dentate gyrus, and this effect could be reversed when animals were on a normal diet for two weeks (99).

It is not known which intracellular compartment containing free or protein-bound zinc is most affected by dietary zinc restriction. Diets completely lacking zinc have limited ability to significantly alter free zinc content such as vesicular zinc in the central nervous system. A zinc-free diet fed to rats for almost three months is required to see any significant change in the zinc content of hippocampal mossy fibers (23, 107). Given the well-documented correlation between zinc restriction and apoptosis and the high metabolic sensitivity of mitochondria, it is possible that zinc sequestered to mitochondrial proteins is most affected.

Epileptic seizures in animals that were fed a zinc-free diet resulted in a smaller number of dying GABAergic interneurons; however, this diet had no effect on the survival rate of pyramidal cells. The diet also failed to alter the maximum seizure severity score and progression of seizures (23). Animals kept on a zinc-free diet develop anorexia and are prone to infections. These changes also need to be taken into consideration when a zinc-free diet is used as an experimental tool to alter zinc homeostasis.

## ZINC AND NEUROPATHOLOGY

### Epilepsy

Mossy fiber sprouting has been observed in human temporal lobe epilepsy and in several animal models of epilepsy (14). Under normal conditions, hippocampal mossy fibers are restricted to the hilus and the stratum lucidum of the CA3 area. However, in epileptic animals and humans, mossy fibers also could be identified in the supragranular layer of the dentate gyrus by Timm staining (45, 101). Reorganization of the mossy fiber system under pathological conditions could contribute to an observed increase in hippocampal zinc levels after kindling (72). Because zinc can modify inhibitory and excitatory synaptic transmission, whether an increased level of zinc is anticonvulsive or proconvulsive is not immediately obvious. Intraperitoneal injection of diethyldithiocarbamate decreased the duration of kindling-induced seizures and electrical afterdischarges, suggesting that zinc could have a proconvulsive effect (33). In contrast, the fact that ZnT3 KO animals are more susceptible to kainate-induced seizures would suggest that zinc is anticonvulsive (21). In the hippocampus, mossy fibers innervate interneurons and pyramidal cells; zinc can modulate both excitatory and inhibitory neurotransmission. Hippocampal interneurons contribute to feedback and feed-forward inhibition. Predicting the role of zinc in excitability is very difficult, if not impossible, given that zinc can potentially act on every synapse of this complex network. This question is further complicated by the apparent lack of information about the exact concentration of zinc in the synaptic cleft and the extracellular space. In order to overcome these issues, we chose to eliminate endogenous zinc from hippocampal slices to study how general network excitability is altered when zinc is chelated. Surprisingly, membrane-impermeable zinc chelation had no significant effect on high-potassium-induced spontaneous burst generation in the CA3 area of the hippocampus (59), suggesting that phasic or tonic zinc release does not alter excitability. In contrast, zinc chelation with the membrane-permeable zinc chelator diethyldithiocarbamate altered the firing threshold of CA3 pyramidal cells. The exact mechanism of this effect is not known. Given that zinc is a coenzyme in more than

300 proteins, the number of potential intracellular mechanisms that could be affected is incredibly high.

As discussed above, GABA receptors with different subunit compositions show different sensitivity to zinc. Under normal conditions, synaptic GABA signals are not influenced by extracellular zinc but are predominantly zinc sensitive in epileptic tissue (36, 93). When zinc was applied extracellularly to in vitro slices from kindled animals (11) or isolated dentate granule cells from pilocarpine-treated rats (36), it depressed GABAergic synaptic signals. The change in zinc sensitivity was believed to be caused by the altered subunit composition of GABAergic receptors in epileptic animals (6). Taken together, these findings indicate that in the epileptic brain, GABA receptors become zinc-sensitive and are blocked by zinc associated to mossy fiber terminals in an activity-dependent manner. This reduced inhibition would contribute to the increased excitability of the dentate gyrus–CA3 network. Because zinc is sequestered to vesicles containing glutamate, zinc needs to travel relatively fast from glutamatergic to the GABAergic synapses in order to alter inhibitory signaling. If this is the case, GABAergic responses evoked with the direct application of GABA agonists should be inhibited when mossy fibers are stimulated. This possibility was tested by Molnár & Nadler (73), and their data showed that mossy fiber activation does not have such an effect, suggesting that endogenous zinc from mossy fibers does not contribute to the zinc-induced "collapse of inhibition" as suggested previously (11).

During epileptic seizures, increased cellular activity leads to excessive glutamate release, which in turn leads to the overactivation of glutamate receptors and causes excitotoxicity and eventually cell death. Extracellularly applied high concentration of zinc was shown to be toxic: Under this condition, zinc can enter the cell and induce mitochondrial dysfunction, leading to apoptosis (17). Zinc as divalent cation can permeate through NMDA, $Ca^{2+}$-permeable AMPA, and kainate receptors (106).

While on the postsynaptic site, zinc accumulation in the cytoplasm of vulnerable neurons was observed; on the presynaptic site, zinc levels decreased following seizures (34). It is tempting to conclude that a decrease in zinc levels on the presynaptic site and an increase in zinc levels on the postsynaptic site are directly related, as zinc would translocate from the pre- to the postsynaptic site. If this is indeed the case, the temporal properties of the observed changes in zinc level should be similar on both sides of the synapse. Decreases in presynaptic zinc levels occur immediately after the onset of seizures (59) (**Figure 1*B–D***) and levels return to normal values a few hours after the offset of seizures (70). On the postsynaptic site, cytosolic zinc levels reach their maximum ~10 hours after seizure initiation in CA3 inhibitory interneurons and ~18 hours later in pyramidal cells (23). It is unlikely that zinc released from the presynaptic terminal would be in "transit" for several hours. In case of direct translocation, extracellular zinc chelation and genetic removal of vesicular zinc should lead to diminished zinc accumulation in vulnerable cells. However, this is not the case. Neither extracellular zinc chelation nor the ablation of vesicular zinc from the mossy fiber terminals in ZnT3 KO animals could prevent zinc accumulation and cell death after seizures (59, 60). These data rather suggest that in epileptic animals zinc is released from intracellular stores in vulnerable cells, such as metallothioneins and mitochondria (56, 91). However, in in vitro slices, spreading depression evoked by high $[K^+]$ puffs was shown to increase intracellular zinc levels. This increase was not observed in ZnT3 KO animals, suggesting that it originated from synaptic vesicles (13).

Zinc has been suggested to play both neuroprotective and neurotoxic roles. Although extracellularly applied zinc was shown to be toxic, zinc chelation or ablation of ZnT3 from synaptic vesicles did not prevent excitotoxic cell death (23, 27, 60). Zinc removal rather

increased the rate of cell death and lowered seizure threshold, suggesting that at physiological concentrations, zinc plays an essential role in maintaining a stable network and preventing cell death. Our data indicate that when zinc concentration is too low, cells lose their ability to activate the antipoptotic pathway and avoid cell death. In contrast, zinc levels that are too high lead to cell death (23). This situation is not that different from what we know about the calcium milieu: Physiological concentration needs to be maintained between normal values, and divergence in either direction from these values will have detrimental effects.

## Alzheimer's Disease

Zinc and other endogenous metal ions were suggested to play a role in Aβ aggregation in brains of patients suffering from Alzheimer's disease. Zinc can trigger the aggregation of synthetic Aβ, and three histidine residues in the N-terminal region were identified as primary metal-binding sites (71). Binding properties of $Zn^{2+}$ and $Cu^{2+}$ suggested that under normal physiological conditions, $Cu^{2+}$ prevents $Zn^{2+}$ binding and hence prevents Aβ aggregation. Increased zinc levels were observed in the brains from Alzheimer's patients (25, 26, 65), suggesting that disturbances in zinc homeostasis could help the development of amyloid plaques. In animal models of Alzheimer's disease, oral treatment with the zinc and copper chelator clioquinol decreased Aβ deposits by ~50% (16). A second generation of Zn/Cu chelator, PBT2, is more effective as an ionophore and has a greater blood-brain permeability. Oral administration of this agent decreased soluble interstitial brain Aβ and improved cognitive performance (2). A safety, tolerability, and efficacy clinical trial for this drug has shown that it is tolerable and safe to use (58). Further studies will be needed to determine its effectiveness in patients suffering from Alzheimer's disease.

## Aging

The high concentration of zinc observed in hippocampal mossy fibers led to the speculation that zinc could play a crucial role in hippocampal information processing and hence in spatial memory formation. Therefore, it was rather unexpected to see that ZnT3 KO animals did not suffer any obvious learning deficits. ZnT3 mice showed normal behavior in the open-field and elevated plus maze tests. These animals exhibited normal learning and memory in the passive avoidance, Morris water maze, and fear conditioning tasks. They also had normal working and reference memory in the radial arm maze (20). However, in aging ZnT3 KO animals, cognitive deficits were reported. At six months of age, ZnT3 KO animals performed significantly worse in the Morris water maze (3). Age-dependent changes were also observed in the barrel cortex of mice: In older animals, activity-dependent changes in zinc levels were less dynamic than in younger animals (9).

# CONCLUSION

The role of endogenous zinc in synaptic transmission still remains an enigma. Since it is not known when and/or if zinc is released from synaptic terminals, and what the concentration of zinc is in the synaptic cleft, the role of endogenous zinc can be studied with either the elimination of zinc from the synapse or with the elimination of specific zinc-binding sites from neurotransmitter receptors. Zinc chelation will affect both phasic and tonic zinc signals and renders data interpretation about the role of vesicular zinc difficult. Transgenic animals lacking a specific zinc transporter could develop compensatory mechanisms, development of conditional KO mice could overcome this problem. After decades of research on the role of zinc in synaptic transmission, we are still waiting for a definite answer. It is hoped that in the near future improved experimental tools will unveil the fundamental role of zinc in synaptic signaling.

## DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## LITERATURE CITED


1. Adamo AM, Zago MP, Mackenzie GG, Aimo L, Keen CL, et al. 2010. The role of zinc in the modulation of neuronal proliferation and apoptosis. *Neurotox. Res.* 17:1–14
2. Adlard PA, Cherny RA, Finkelstein DI, Gautier E, Robb E, et al. 2008. Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Abeta. *Neuron* 59:43–55
3. Adlard PA, Parncutt JM, Finkelstein DI, Bush AI. 2010. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? *J. Neurosci.* 30:1631–36
4. Armstrong JN, Saganich MJ, Xu NJ, Henkemeyer M, Heinemann SF, Contractor A. 2006. B-ephrin reverse signaling is required for NMDA-independent long-term potentiation of mossy fibers in the hippocampus. *J. Neurosci.* 26:3474–81
5. Bellocchio EE, Reimer RJ, Fremeau RT, Edwards RH. 2000. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* 289:957–60
6. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. 1998. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. *Nat. Med.* 4:1166–72
7. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. 2001. gamma-Aminobutyric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J. Neurochem.* 77:1266–78
8. Brown CE, Dyck RH. 2002. Rapid, experience-dependent changes in levels of synaptic zinc in primary somatosensory cortex of the adult mouse. *J. Neurosci.* 22:2617–25
9. Brown CE, Dyck RH. 2003. Experience-dependent regulation of synaptic zinc is impaired in the cortex of aged mice. *Neuroscience* 119:795–801
10. Brown CE, Dyck RH. 2005. Modulation of synaptic zinc in barrel cortex by whisker stimulation. *Neuroscience* 134:355–59
11. Buhl EH, Otis TS, Mody I. 1996. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science* 271:369–73
12. Caldwell DF, Oberleas D, Clancy JJ, Prasad AS. 1970. Behavioral impairment in adult rats following acute zinc deficiency. *Proc. Soc. Exp. Biol. Med.* 133:1417–21
13. Carter RE, Aiba I, Dietz RM, Sheline CT, Shuttleworth CW. 2011. Spreading depression and related events are significant sources of neuronal $Zn^{2+}$ release and accumulation. *J. Cereb. Blood Flow Metab.* 31:1073–84
14. Cavazos JE, Golarai G, Sutula TP. 1991. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *J. Neurosci.* 11:2795–803
15. Chen N, Moshaver A, Raymond LA. 1997. Differential sensitivity of recombinant N-methyl-D-aspartate receptor subtypes to zinc inhibition. *Mol. Pharmacol.* 51:1015–23
16. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, et al. 2001. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30:665–76
17. Choi DW, Koh JY. 1998. Zinc and brain injury. *Annu. Rev. Neurosci.* 21:347–75
18. Choi YB, Lipton SA. 1999. Identification and mechanism of action of two histidine residues underlying high-affinity $Zn^{2+}$ inhibition of the NMDA receptor. *Neuron* 23:171–80
19. Christine CW, Choi DW. 1990. Effect of zinc on NMDA receptor-mediated channel currents in cortical neurons. *J. Neurosci.* 10:108–16
20. Cole TB, Martyanova A, Palmiter RD. 2001. Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. *Brain Res.* 891:253–65
21. Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD. 2000. Seizures and neuronal damage in mice lacking vesicular zinc. *Epilepsy Res.* 39:153–69

22. Contractor A, Rogers C, Maron C, Henkemeyer M, Swanson GT, Heinemann SF. 2002. Trans-synaptic Eph receptor-ephrin signaling in hippocampal mossy fiber LTP. *Science* 296:1864–69
23. Côté A, Chiasson M, Peralta MR, Lafortune K, Pellegrini L, Tóth K. 2005. Cell type-specific action of seizure-induced intracellular zinc accumulation in the rat hippocampus. *J. Physiol.* 566:821–37
24. Danglot L, Galli T. 2007. What is the function of neuronal AP-3? *Biol. Cell* 99:349–61
25. Danscher G, Jensen KB, Frederickson CJ, Kemp K, Andreasen A, et al. 1997. Increased amount of zinc in the hippocampus and amygdala of Alzheimer's diseased brains: a proton-induced X-ray emission spectroscopic analysis of cryostat sections from autopsy material. *J. Neurosci. Methods* 76:53–59
26. Deibel MA, Ehmann WD, Markesbery WR. 1996. Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. *J. Neurol. Sci.* 143:137–42
27. Domínguez MI, Blasco-Ibáñez JM, Crespo C, Marqués-Marí AI, Martínez-Guijarro FJ. 2003. Zinc chelation during non-lesioning overexcitation results in neuronal death in the mouse hippocampus. *Neuroscience* 116:791–806
28. Draguhn A, Verdorn TA, Ewert M, Seeburg PH, Sakmann B. 1990. Functional and molecular distinction between recombinant rat GABAA receptor subtypes by $Zn^{2+}$. *Neuron* 5:781–88
29. Dreixler JC, Leonard JP. 1994. Subunit-specific enhancement of glutamate receptor responses by zinc. *Brain Res. Mol. Brain Res.* 22:144–50
30. Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. 1983. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. I. Impaired acquisition of granule cells. *Brain Res.* 271:217–26
31. Farrant M, Nusser Z. 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. *Nat. Rev. Neurosci.* 6:215–29
32. Fayyazuddin A, Villarroel A, Le Goff A, Lerma J, Neyton J. 2000. Four residues of the extracellular N-terminal domain of the NR2A subunit control high-affinity $Zn^{2+}$ binding to NMDA receptors. *Neuron* 25:683–94
33. Foresti ML, Arisi GM, Fernandes A, Tilelli CQ, Garcia-Cairasco N. 2008. Chelatable zinc modulates excitability and seizure duration in the amygdala rapid kindling model. *Epilepsy Res.* 79:166–72
34. Frederickson CJ, Hernandez MD, McGinty JF. 1989. Translocation of zinc may contribute to seizure-induced death of neurons. *Brain Res.* 480:317–21
35. Fukushima T, Shingai R, Ogurusu T, Ichinose M. 2003. Inhibition of willardiine-induced currents through rat GluR6/KA-2 kainate receptor channels by zinc and other divalent cations. *Neurosci. Lett.* 349:107–10
36. Gibbs JW, Shumate MD, Coulter DA. 1997. Differential epilepsy-associated alterations in postsynaptic GABA(A) receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.* 77:1924–38
37. Gielen M, Le Goff A, Stroebel D, Johnson JW, Neyton J, Paoletti P. 2008. Structural rearrangements of NR1/NR2A NMDA receptors during allosteric inhibition. *Neuron* 57:80–93
38. Gielen M, Siegler Retchless B, Mony L, Johnson JW, Paoletti P. 2009. Mechanism of differential control of NMDA receptor activity by NR2 subunits. *Nature* 459:703–7
39. Golub MS, Keen CL, Gershwin ME, Hendrickx AG. 1995. Developmental zinc deficiency and behavior. *J. Nutr.* 125:2263–71S
40. Golub MS, Takeuchi PT, Keen CL, Gershwin ME, Hendrickx AG, Lonnerdal B. 1994. Modulation of behavioral performance of prepubertal monkeys by moderate dietary zinc deprivation. *Am. J. Clin. Nutr.* 60:238–43
41. Hirzel K, Müller U, Latal AT, Hülsmann S, Grudzinska A, et al. 2006. Hyperekplexia phenotype of glycine receptor alpha1 subunit mutant mice identifies $Zn^{2+}$ as an essential endogenous modulator of glycinergic neurotransmission. *Neuron* 52:679–90
42. Hoo KH, Nutt SL, Fletcher EJ, Elliott CE, Korczak B, et al. 1994. Functional expression and pharmacological characterization of the human EAA4 (GluR6) glutamate receptor: a kainate selective channel subunit. *Receptors Channels* 2:327–37
43. Huang YZ, Pan E, Xiong ZQ, McNamara JO. 2008. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron* 57:546–58
44. Hurley LS, Swenerton H. 1966. Congenital malformations resulting from zinc deficiency in rats. *Proc. Soc. Exp. Biol. Med.* 123:692–96

45. Isokawa M, Levesque MF, Babb TL, Engel J. 1993. Single mossy fiber axonal systems of human dentate granule cells studied in hippocampal slices from patients with temporal lobe epilepsy. *J. Neurosci.* 13:1511–22
46. Izumi Y, Auberson YP, Zorumski CF. 2006. Zinc modulates bidirectional hippocampal plasticity by effects on NMDA receptors. *J. Neurosci.* 26:7181–88
47. Janes PW, Saha N, Barton WA, Kolev MV, Wimmer-Kleikamp SH, et al. 2005. Adam meets Eph: An ADAM substrate recognition module acts as a molecular switch for ephrin cleavage in trans. *Cell* 123:291–304
48. Kamiya H, Shinozaki H, Yamamoto C. 1996. Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. *J. Physiol.* 493(Pt. 2):447–55
49. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, et al. 1998. Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. *Neuron* 21:111–22
50. Kay AR. 2003. Evidence for chelatable zinc in the extracellular space of the hippocampus, but little evidence for synaptic release of Zn. *J. Neurosci.* 23:6847–55
51. Kay AR. 2004. Detecting and minimizing zinc contamination in physiological solutions. *BMC Physiol.* 4:4
52. Kay AR. 2006. Imaging synaptic zinc: promises and perils. *Trends Neurosci.* 29:200–6
53. Kay AR, Tóth K. 2008. Is zinc a neuromodulator? *Sci. Signal.* 1:re3
54. Keller KA, Grider A, Coffield JA. 2001. Age-dependent influence of dietary zinc restriction on short-term memory in male rats. *Physiol. Behav.* 72:339–48
55. Kodirov SA, Takizawa S, Joseph J, Kandel ER, Shumyatsky GP, Bolshakov VY. 2006. Synaptically released zinc gates long-term potentiation in fear conditioning pathways. *Proc. Natl. Acad. Sci. USA* 103:15218–23
56. Land PW, Aizenman E. 2005. Zinc accumulation after target loss: an early event in retrograde degeneration of thalamic neurons. *Eur. J. Neurosci.* 21:647–57
57. Land PW, Akhtar ND. 1999. Experience-dependent alteration of synaptic zinc in rat somatosensory barrel cortex. *Somatosens. Mot. Res.* 16:139–50
58. Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, et al. 2008. Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *Lancet Neurol.* 7:779–86
59. Lavoie N, Peralta MR, Chiasson M, Lafortune K, Pellegrini L, et al. 2007. Extracellular chelation of zinc does not affect hippocampal excitability and seizure-induced cell death in rats. *J. Physiol.* 578:275–89
60. Lee JY, Cole TB, Palmiter RD, Koh JY. 2000. Accumulation of zinc in degenerating hippocampal neurons of ZnT3-null mice after seizures: evidence against synaptic vesicle origin. *J. Neurosci.* 20:RC79
61. Legendre P, Westbrook GL. 1990. The inhibition of single N-methyl-D-aspartate-activated channels by zinc ions on cultured rat neurones. *J. Physiol.* 429:429–49
62. Li Y, Calfa G, Inoue T, Amaral MD, Pozzo-Miller L. 2010. Activity-dependent release of endogenous BDNF from mossy fibers evokes a TRPC3 current and $Ca^{2+}$ elevations in CA3 pyramidal neurons. *J. Neurophysiol.* 103:2846–56
63. Li Y, Hough CJ, Frederickson CJ, Sarvey JM. 2001. Induction of mossy fiber –> Ca3 long-term potentiation requires translocation of synaptically released $Zn^{2+}$. *J. Neurosci.* 21:8015–25
64. Lin DD, Cohen AS, Coulter DA. 2001. Zinc-induced augmentation of excitatory synaptic currents and glutamate receptor responses in hippocampal CA3 neurons. *J. Neurophysiol.* 85:1185–96
65. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. 1998. Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurol. Sci.* 158:47–52
66. Low CM, Zheng F, Lyuboslavsky P, Traynelis SF. 2000. Molecular determinants of coordinated proton and zinc inhibition of N-methyl-D-aspartate NR1/NR2A receptors. *Proc. Natl. Acad. Sci. USA* 97:11062–67
67. Ma JY, Zhao ZQ. 2001. The effects of $Zn^{2+}$ on long-term potentiation of C fiber-evoked potentials in the rat spinal dorsal horn. *Brain Res. Bull.* 56:575–79
68. Mayer ML, Vyklicky L. 1989. The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. *J. Physiol.* 415:351–65

69. Mayer ML, Vyklicky L, Westbrook GL. 1989. Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *J. Physiol.* 415:329–50
70. Mitsuya K, Nitta N, Suzuki F. 2009. Persistent zinc depletion in the mossy fiber terminals in the intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. *Epilepsia* 50:1979–90
71. Miura T, Suzuki K, Kohata N, Takeuchi H. 2000. Metal binding modes of Alzheimer's amyloid beta-peptide in insoluble aggregates and soluble complexes. *Biochemistry* 39:7024–31
72. Mody I, Miller JJ. 1985. Levels of hippocampal calcium and zinc following kindling-induced epilepsy. *Can. J. Physiol. Pharmacol.* 63:159–61
73. Molnár P, Nadler JV. 2001a. Lack of effect of mossy fiber-released zinc on granule cell GABA(A) receptors in the pilocarpine model of epilepsy. *J. Neurophysiol.* 85:1932–40
74. Molnár P, Nadler JV. 2001b. Synaptically-released zinc inhibits N-methyl-D-aspartate receptor activation at recurrent mossy fiber synapses. *Brain Res.* 910:205–7
75. Mott DD, Benveniste M, Dingledine RJ. 2008. pH-Dependent inhibition of kainate receptors by zinc. *J. Neurosci.* 28:1659–71
76. Nakashima AS, Dyck RH. 2010. Dynamic, experience-dependent modulation of synaptic zinc within the excitatory synapses of the mouse barrel cortex. *Neuroscience* 170:1015–19
77. Nusser Z, Sieghart W, Somogyi P. 1998. Segregation of different GABAA receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J. Neurosci.* 18:1693–703
78. Palmiter RD, Cole TB, Quaife CJ, Findley SD. 1996. ZnT-3, a putative transporter of zinc into synaptic vesicles. *Proc. Natl. Acad. Sci. USA* 93:14934–39
79. Paoletti P, Ascher P, Neyton J. 1997. High-affinity zinc inhibition of NMDA NR1-NR2A receptors. *J. Neurosci.* 17:5711–25
80. Paoletti P, Perin-Dureau F, Fayyazuddin A, Le Goff A,Callebaut I, Neyton J. 2000. Molecular organization of a zinc binding n-terminal modulatory domain in a NMDA receptor subunit. *Neuron* 28:911–25
81. Paoletti P, Vergnano AM, Barbour B, Casado M. 2009. Zinc at glutamatergic synapses. *Neuroscience* 158:126–36
82. Pérez-Clausell J, Danscher G. 1985. Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. *Brain Res.* 337:91–98
83. Peters S, Koh J, Choi DW. 1987. Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science* 236:589–93
84. Rachline J, Perin-Dureau F, Le Goff A, Neyton J, Paoletti P. 2005. The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. *J. Neurosci.* 25:308–17
85. Ruiz A, Walker MC, Fabian-Fine R, Kullmann DM. 2004. Endogenous zinc inhibits GABA(A) receptors in a hippocampal pathway. *J. Neurophysiol.* 91:1091–96
86. Salazar G, Craige B, Love R, Kalman D, Faundez V. 2005. Vglut1 and ZnT3 co-targeting mechanisms regulate vesicular zinc stores in PC12 cells. *J. Cell Sci.* 118:1911–21
87. Salazar G, Love R, Styers ML, Werner E, Peden A, et al. 2004. AP-3-dependent mechanisms control the targeting of a chloride channel (ClC-3) in neuronal and non-neuronal cells. *J. Biol. Chem.* 279:25430–39
88. Salazar G, Love R, Werner E, Doucette MM, Cheng S, et al. 2004. The zinc transporter ZnT3 interacts with AP-3 and it is preferentially targeted to a distinct synaptic vesicle subpopulation. *Mol. Biol. Cell* 15:575–87
89. Schenck S, Wojcik SM, Brose N, Takamori S. 2009. A chloride conductance in VGLUT1 underlies maximal glutamate loading into synaptic vesicles. *Nat. Neurosci.* 12:156–62
90. Scheuber A, Rudge R, Danglot L, Raposo G, Binz T, et al. 2006. Loss of AP-3 function affects spontaneous and evoked release at hippocampal mossy fiber synapses. *Proc. Natl. Acad. Sci. USA* 103:16562–67
91. Sensi SL, Ton-That D, Sullivan PG, Jonas EA, Gee KR, et al. 2003. Modulation of mitochondrial function by endogenous $Zn^{2+}$ pools. *Proc. Natl. Acad. Sci. USA* 100:6157–62
92. Shen Y, Yang XL. 1999. Zinc modulation of AMPA receptors may be relevant to splice variants in carp retina. *Neurosci. Lett.* 259:177–80
93. Shumate MD, Lin DD, Gibbs JW, Holloway KL, Coulter DA. 1998. GABA(A) receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. *Epilepsy Res.* 32:114–28
94. Smart TG, Constanti A. 1982. A novel effect of zinc on the lobster muscle GABA receptor. *Proc. R. Soc. Lond. B Biol. Sci.* 215:327–41

95. Smart TG, Constanti A. 1990. Differential effect of zinc on the vertebrate GABAA-receptor complex. *Br. J. Pharmacol.* 99:643–54
96. Smart TG, Moss SJ, Xie X, Huganir RL. 1991. GABAA receptors are differentially sensitive to zinc: dependence on subunit composition. *Br. J. Pharmacol.* 103:1837–39
97. Stobrawa SM, Breiderhoff T, Takamori S, Engel D, Schweizer M, et al. 2001. Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron* 29:185–96
98. Stoltenberg M, Nejsum LN, Larsen A, Danscher G. 2004. Abundance of zinc ions in synaptic terminals of mocha mutant mice: zinc transporter 3 immunohistochemistry and zinc sulphide autometallography. *J. Mol. Histol.* 35:141–45
99. Suh SW, Won SJ, Hamby AM, Yoo BH, Fan Y, et al. 2009. Decreased brain zinc availability reduces hippocampal neurogenesis in mice and rats. *J. Cereb. Blood Flow Metab.* 29:1579–88
100. Sun Y, Jiang XD, Liu X, Gong HQ, Liang PJ. 2010. Synaptic contribution of $Ca^{2+}$-permeable and $Ca^{2+}$-impermeable AMPA receptors on isolated carp retinal horizontal cells and their modulation by $Zn^{2+}$. *Brain Res.* 1317:60–68
101. Sutula T, He XX, Cavazos J, Scott G. 1988. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* 239:1147–50
102. Tóth K, Suares G, Lawrence JJ, Philips-Tansey E, McBain CJ. 2000. Differential mechanisms of transmission at three types of mossy fiber synapse. *J. Neurosci.* 20:8279–89
103. Traynelis SF, Burgess MF, Zheng F, Lyuboslavsky P, Powers JL. 1998. Control of voltage-independent zinc inhibition of NMDA receptors by the NR1 subunit. *J. Neurosci.* 18:6163–75
104. Vogt K, Mellor J, Tong G, Nicoll R. 2000. The actions of synaptically released zinc at hippocampal mossy fiber synapses. *Neuron* 26:187–96
105. Wei W, Zhang N, Peng Z, Houser CR, Mody I. 2003. Perisynaptic localization of delta subunit-containing GABA(A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J. Neurosci.* 23:10650–61
106. Weiss JH, Sensi SL. 2000. $Ca^{2+}$-$Zn^{2+}$ permeable AMPA or kainate receptors: possible key factors in selective neurodegeneration. *Trends Neurosci.* 23:365–71
107. Wensink J, Lenglet WJ, Vis RD, Van den Hamer CJ. 1987. The effect of dietary zinc deficiency on the mossy fiber zinc content of the rat hippocampus. A microbeam PIXE study. Particle Induced X-Ray Emission. *Histochemistry* 87:65–69
108. Westbrook GL, Mayer ML. 1987. Micromolar concentrations of $Zn^{2+}$ antagonize NMDA and GABA responses of hippocampal neurons. *Nature* 328:640–43
109. Williams K. 1996. Separating dual effects of zinc at recombinant N-methyl-D-aspartate receptors. *Neurosci. Lett.* 215:9–12
110. Wolfsberg TG, Primakoff P, Myles DG, White JM. 1995. ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: multipotential functions in cell-cell and cell-matrix interactions. *J. Cell Biol.* 131:275–78
111. Xie X, Hider RC, Smart TG. 1994. Modulation of GABA-mediated synaptic transmission by endogenous zinc in the immature rat hippocampus in vitro. *J. Physiol.* 478(Pt. 1):75–86
112. Yeckel MF, Kapur A, Johnston D. 1999. Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. *Nat. Neurosci.* 2:625–33
113. Zalutsky RA, Nicoll RA. 1990. Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248:1619–24

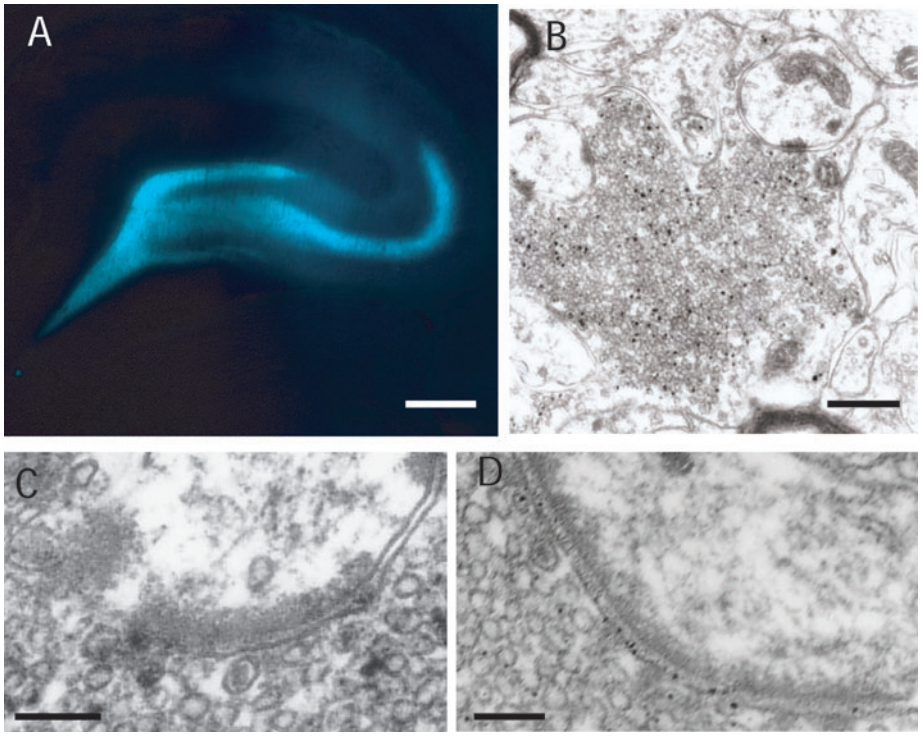


**Figure 1**

Distribution of zinc in the hippocampus. (*A*) TSQ labeling in the hippocampus clearly identifies the mossy fiber system. (*B*) Electron micrograph of a mossy fiber terminal stained with Timm staining. Small black particles indicate the location of chelatable zinc in the presynaptic terminal. (*C* and *D*) Higher-magnification electron micrographs show the change in the distribution of zinc after increased synaptic activity. (*C*) In control animals, zinc staining is restricted to the presynaptic terminal. (*D*) After KA injection, zinc staining appears on the presynaptic membrane surface, indicating increased exocytosis of zinc-containing vesicles. (*C* and *D* from Reference 59, with permission.)

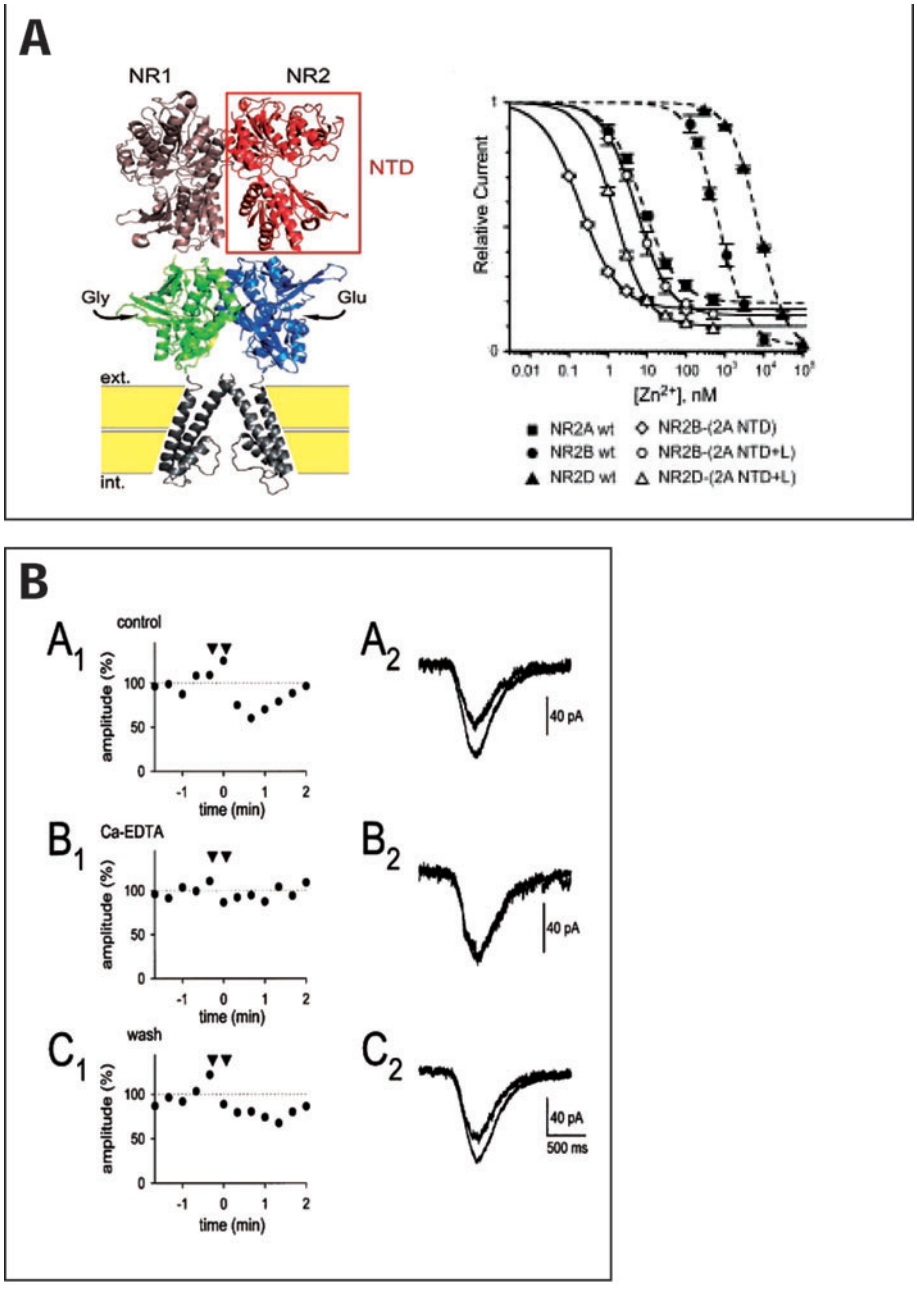


**Figure 2**

Zinc and N-methyl-D-aspartic acid (NMDA) receptor function. (*A*) NMDA receptor structure showing the NR2 amino-terminal domain (NTD) region, which is the target for allosteric inhibitors such as zinc. NR2A-NTD forms a high-affinity inhibitory site. (From Reference 38 with permission.) (*B*) Activity-dependent modulation of NMDA responses at the hippocampal mossy fiber terminals. Iontophoretically applied glutamate responses recorded in CA3 pyramidal cells were inhibited when mossy fiber pathway was stimulated (*arrows*) ($A_1$, $A_2$ and $C_1$, $C_2$); this effect was blocked with calcium disodium ethylenediamine tetraacetate (CaEDTA) ($B_1$, $B_2$). (From Reference 104, with permission.)

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# Contents

**Indexes**



**Errata**

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