Zinc and Health: Current Status and Future Directions

Importance of Zinc in the Central Nervous System: The Zinc-Containing Neuron[1]

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ABSTRACT Zinc is essential to the structure and function of myriad proteins, including regulatory, structural and enzymatic. It is estimated that up to 1% of the human genome codes for zinc finger proteins. In the central nervous system, zinc has an additional role as a neurosecretory product or cofactor. In this role, zinc is highly concentrated in the synaptic vesicles of a specific contingent of neurons, called "zinc-containing" neurons. Zinc-containing neurons are a subset of glutamatergic neurons. The zinc in the vesicles probably exceeds 1 mmol/L in concentration and is only weakly coordinated with any endogenous ligand. Zinc-containing neurons are found almost exclusively in the forebrain, where in mammals they have evolved into a complex and elaborate associational network that interconnects most of the cerebral cortices and limbic structures. Indeed, one of the intriguing aspects of these neurons is that they compose somewhat of a chemospecific "private line" of the mammalian cerebral cortex. The present review outlines (*1*) the methods used to discover, define and describe zinc-containing neurons; (*2*) the neuroarchitecture and synaptology of zinc-containing neural circuits; (*3*) the physiology of regulated vesicular zinc release; (*4*) the "life cycle" and molecular biology of vesicular zinc; (*5*) the importance of synaptically released zinc in the normal and pathological processes of the cerebral cortex; and (*6*) the role of specific and nonspecific stressors in the release of zinc. J. Nutr. 130: 1471S—1483S, 2000.

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Brief History and Overview of Zinc-Containing Neurons

It has been thirty-one years since Finn-Mogens Haug (1967) discovered the first zinc-containing presynaptic vesicles. Although it is often overlooked, Haug actually went far beyond merely describing the zinc-rich hippocampal mossy fibers: he also described in elegant detail the complete zinc-containing circuitry of the hippocampal formation, temporal lobe and, in fact, the entire telencephalon (Haug 1975). Moreover, the Aarhus group led by G. Danscher and Haug also described the rapid release of zinc from mossy-fiber boutons after surgical transection and the physiological and histological effects of zinc chelators on zinc-containing neurons (e.g. Danscher et al. 1973, 1975).

In the decades since the pioneering work of Haug and Danscher, our knowledge of zinc-containing neurons has expanded dramatically. For one thing, it is (mercifully) no longer necessary to refer to the neurons with tongue-twisters like "neurons positive for Timm's-stainable metals." This is because there is no longer doubt that the metal that can be stained in the axonal boutons of the mammalian forebrain is zinc (Danscher 1984, 1996, Danscher et al. 1985, Frederickson et al. 1987, 1992). Furthermore, the anatomical organization of the zinc-containing circuitry that was sketched in the 1970s and 1980s by the Aarhus group is gradually being reconfirmed and drawn in fine detail (and extended to the human brain) with powerful new staining methods, "autometallographic" and zinc-specific retrograde transport methods (Casanovas-Aguilar et al. 1995, Christensen et al. 1992, Danscher et al. 1997a,b, Dyck et al. 1993, Frederickson 1989, Frederickson et al. 1992, Long and Frederickson 1994, Mandava et al. 1993) (**Figs. 1-3**).

The molecular biology of zinc sequestering and pumping into secretory vesicles emerging from the Golgi apparatus is

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[3] Abbreviations used: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABA, γ-aminobutyric acid; KA, kainic acid; NMDA, *N*-methyl-D-aspartate; MT, metallothionein; ZnT, zinc transporter.

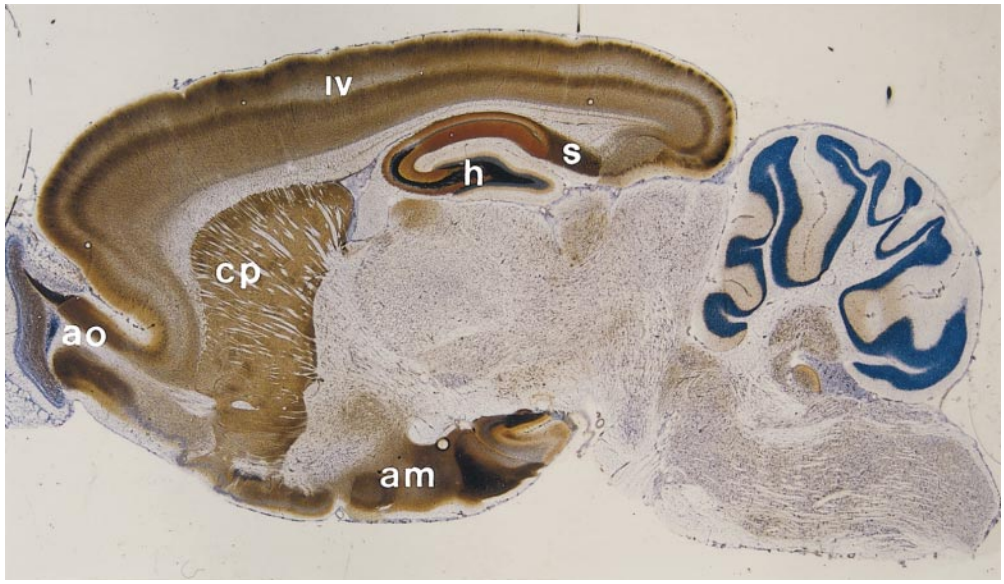

FIGURE 1 Saggital section of rat brain after autometallographic staining. The tan-brown-black staining is the silver staining of zinc by the Timm-Danscher method. The blue is Nissl staining. With use of the electron microscope, all of the Timm-Danscher staining proves to be in the neuropil, in the presynaptic vesicles of gluzinergic neurons. Note the dense staining in the subiculum (s) hilus of dentate gyrus (h) and in the mossy fibers. The accessory olfactory bulb (ao) has substantial gluzinergic innervation, whereas the olfactory bulb proper has very little. The neuropil staining in the caudatoputamen (cp) originates from cortical and amygdalar (am) neurons.

also coming into focus. Proteins and genes specific for sequestering brain zinc and for pumping zinc into vesicles are identified, sequenced and cloned (Aschner et al. 1997, Erickson et al. 1997, Palmiter et al. 1996a, 1996b) (Fig. 6). At the distal end of the neuron (the bouton), the details of zinc storage in the vesicle, of exocytotic release and of reuptake are gradually being revealed as well (Colvin 1998a, 1998b, Wenzel et al. 1997). Finally, across on the postsynaptic side of the synapse, zinc-specific recognition sites that modulate the responsiveness of both excitatory and inhibitory amino acid receptors have been identified and characterized (Frederickson, 1989, Smart et al. 1994) as having transmembrane channels that admit zinc ions into the cytosol of certain postsynaptic neurons under specific conditions (Yin et al. 1998).

Despite the advances in descriptive phenomenology, it is still unknown what unique and particular function is subserved

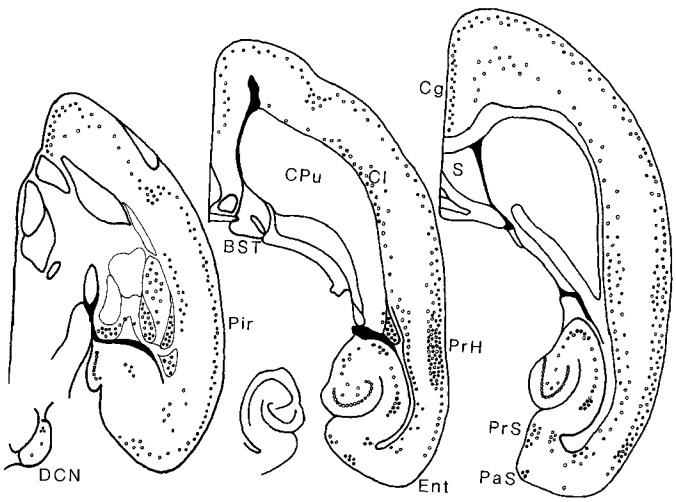

FIGURE 2 Schematic drawing of the distribution of gluzinergic somata. Dots indicate somata and solid dots indicate heavier concentrations in the rat brain. Three of the heaviest concentrations of gluzinergic cells of origin are the perirhinal cortex (PrH), the subjacent lateral amygdala and the prosubiculum (PrS). The cingulate cortex (Cg) and claustrum (Cl) also have abundant somata. Note the complete absence of gluzinergic somata in layer V of isocortex and in the caudatoputamen (CPu). DCN = dorsal cochlear nucleus, Pir = Piriform cortex, BST = bed nucleus of the stria terminalis, Ent = entorhinal cortex, PaS = pana subiculum. S = subiculum.

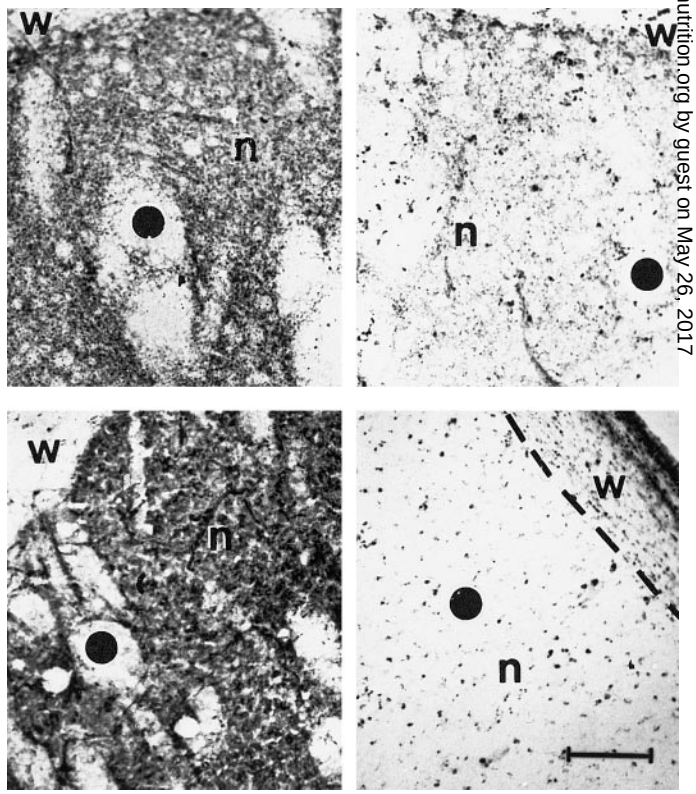

FIGURE 3 Origin of the zinc-containing innervation of the caudatoputamen (striatum). Coronal sections from the intact (*left panels*) and partially denervated (*right panels*) strata of two rats. Normal gluzinergic bouton staining (Timm-Danscher method) is seen throughout the neuropil (n) but not in the axon fascicles (dots) on the left. Cortical white matter (w) is unstained in all. The denervation that caused the loss of boutons on the left in both rats was the complete ipsilateral decortication, combined with transection of the corpus callosum. Note the loss of the zinc-containing boutons.

by the zinc-containing neuronal circuitry of the brain. Because one could say the same thing about neurons defined by, for example, their serotonin content or their histamine, neuropeptide Y, or cholecystokinin content, this fact should not embarrass the student of zinc-containing neurons. Nonetheless, the question of why zinc is richly concentrated in certain neuronal vesicles remains one of the minor mysteries (for some of us, one of the "major mysteries") of neurobiology.

It appears that all zinc-containing neurons are glutamatergic and that only some glutamatergic neurons are zinc containing. This relationship points toward a role of zinc in glutamate storage, release or reuptake, or in modulation of glutamate receptors. Because the vast preponderance of glutamatergic neurons that are zinc containing are in the cerebral cortex and amygdala and the vast proportion of those that are non—zinc containing are subcortical or spinal, many of us have been tempted to speculate (with admittedly scanty evidence) that the special importance of zinc in glutamatergic synaptic function might be related to the cognitive and mnemonic operations unique to the cerebrum (Frederickson et al.1990).

As is often true in medical biology, it is easier to list pathological symptoms of vesicular zinc dysregulation than to state the normal, physiological functions of vesicular zinc. Thus, there is abundant evidence that zinc might be a contributing cause of neuronal injury in brain diseases or injuries that involve excitotoxic mechanisms of neuron injury (Choi 1996, Frederickson et al. 1988). Specifically, the evidence suggests that during ischemia (stroke), seizures or mechanical head injury, there is a released "flood" of zinc from boutons that (*1*) depletes the boutons of zinc and (*2*) allows toxic excesses of zinc to enter postsynaptic neurons, causing injury or death. This "zinc translocation" (Frederickson et al. 1988) is arguably among the leading preventable causes of neuronal injury in adult men and women (Choi 1996, Choi and Koh 1998).

Still, the involvement of zinc in excitotoxicity does not explain why zinc is present in boutons in the first place or why it is released in a regulated fashion. The fact that synaptically released zinc enters postsynaptic neurons, however, raises the intriguing possibility that one of the physiological functions of zinc in the normal brain may actually involve the translocation from one (presynaptic) neuron into the next (postsynaptic) neuron, as a novel, orthograde, transcellular messenger.

Zinc-Containing Neurons: Definition and Methods

Zinc-containing neurons are defined as neurons that sequester weakly bound (histochemically stainable) zinc in the vesicles of their presynaptic boutons (Frederickson 1989, Frederickson and Danscher 1990). It now appears that the zinc accumulates in these vesicles because of the presence of a zinc-specific pump called zinc transporter-3 (ZnT-3)[3] (Fig. 6) that is itself localized on the membranes of the vesicles of these same neurons (Palmiter et al. 1996b, Wenzel et al. 1997). Indeed, zinc-containing neurons may eventually come to be defined by the presence of the ZnT-3 protein in their vesicles (but see later).

It is worth noting in passing that the total amount of brain zinc that is concentrated in the vesicles of the zinc-containing neurons is quite small, probably $< 5\%$ of the total zinc in the brain (Frederickson 1989). This is rather like the situation with glutamate: only a trivial quantity of glutamate is actually sequestered in the vesicles of glutamatergic neurons, with the remainder being present as a constituent of peptides and proteins. Needless to say, the fact that the amount of stored material is small does not make either vesicular glutamate or vesicular zinc unimportant. However, it is tactically important for investigators to bear this in mind. Instrumental bulk assays are almost certainly doomed to failure when one is attempting to detect changes in the miniscule fraction of zinc that is in the vesicles, compared with the larger fraction of protein-bound material in whole brain.

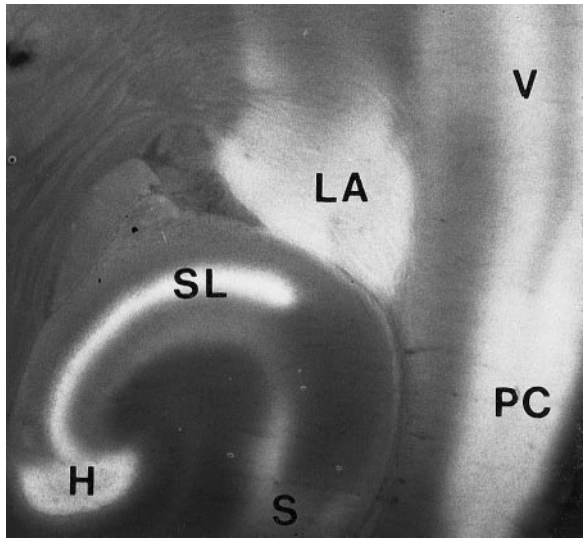

FIGURE 4 Florescence image for zinc after TSQ staining. TSQ fluorescent staining, which is a histochemical stain for histological sections, shows the location of gluzinergic boutons in the neuropil. The perirhinal cortex (PC), hilus of the dentate gyrus (H), stratum lucidum (SL) and lateral amygdala (LA) are all densely innervated with the gluzinergic boutons. Layer V (V) of isocortex is also densely innervated. The subiculum in this image (S) is poorly illuminated by the exciter beam.

Although the vesicular zinc is a small fraction of total zinc in the brain, it constitutes virtually 100% of the histochemically reactive zinc in the brain (Danscher 1985, 1986, 1996, Perez-Clausell and Danscher 1985) (**Figs. 4, 5, 6**). This is a critically important datum: there is no histochemically reactive zinc anywhere but in the secretory vesicles of zinc-containing boutons (or en route in their axons). *Any staining for zinc in the nucleus, perikaryon or dendrites of a neuron in the brain indicates either cell injury or artifact.* This fact is illustrated in the accompanying figures (Figs. 4, 5, 6) that show that staining for zinc occurs only in the axonal boutons of the zinc-containing neurons of the brain.

The choice of methods used to demonstrate the zinc-containing boutons is critical. It is all too easy to lose staining and to create artifactual staining by mishandling the tissue (Danscher 1996, Danscher et al. 1997, Frederickson et al. 1987a, Suh et al. 1999). For example, almost any form of fixation masks subsequent staining of boutons, causing less dense pathways to completely fail to stain and, with prolonged hard fixation, causing all staining to fail. On the other hand, treating tissue with harsh and aggressive reagents or simply leaving the tissue to decompose in situ after death for a dozen hours or so reliably causes (*1*) staining by some methods to be completely blocked and (*2*) staining by other methods to be dramatically amplified (Frederickson et al. 1987a). Finally, even with freshly cut, rapidly frozen, unfixed cryostat sections of brain tissue, staining reagents that cannot penetrate the lipid membranes of (largely intact) boutons and vesicles will still fail to show the zinc in the axonal boutons (Suh et al. 2000).

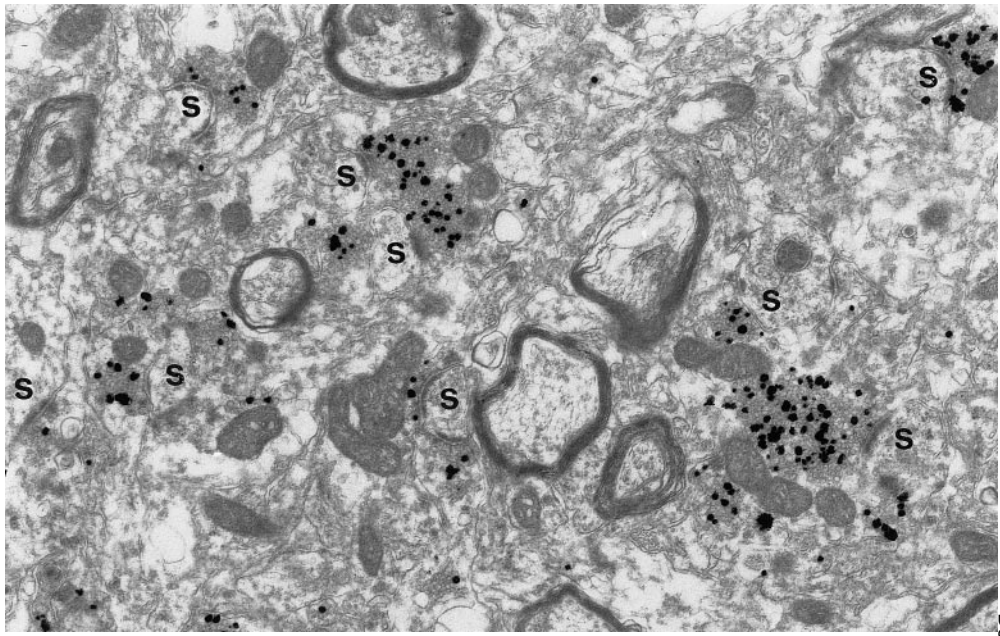

FIGURE 5 Zinc-containing (gluzinergic) boutons of the human cerebral cortex. The postmortem gas-AMG method of Danscher was used to show the zinc (silver grains) in the boutons of human. Note that virtually all staining is in type I boutons apposed to asymmetric synaptic specializations, in spines (s). (Courtesy of G. Danscher.)

Because the ZnT-3 protein is evidently present in all zinc-containing vesicles, one could define and identify zinc-containing neurons by the immunohistochemical loccalization of the transporter, as well as by zinc histochemistry. The electron microscopic survey of mossy-fiber neuropil in the rat indicates that virtually all boutons have both ZnT-3 transporters as well as zinc, indicating a possible equivalence of the two markers (Wenzel et al. 1997) (Fig. 6). However, whether there are neurons that express the ZnT-3 gene but do not concentrate zinc in their vesicles (due to post-translational modification of the protein) is not determined. In fact, the mouse hippocampus shows rather striking regional differences (especially in the lateral perforant pathway and the direct, temporoammonic pathway) when stained for ZnT-3 in boutons compared with the staining for zinc in boutons (Palmiter et al. 1996, Wenzel et al. 1997).

In addition to identifying the boutons, it is possible to selectively label and identify the somata of zinc-containing neurons. Thus, if one labels vesicular zinc by intravital precipitation of the zinc with intravital selenium and then waits 24 h for retrograde transport of the ZnSe, the neuronal somata that can then be visualized by ZnSe histochemistry (the cells of origin of the ZnSe-labeled boutons) are the zinc-containing somata (Danscher 1984) (**Figs.** 2, **7**). When the selenium is administered intraperitoneally, the labeling is weak and capricious, resulting in rather massive false-negative errors (Slomianka et al. 1990). On the other hand, when the selenium is infused directly into the neuropil of interest, the zinc-containing neurons with terminals in the infused region are reliably and selectively labeled, with no known false-positives or false-negatives (Frederickson, 1990, Howell and Frederickson 1989). Just as zinc-containing boutons can be potentially identified by ZnT-3 immunochemistry, the zinc-containing somata should be identifiable by visualization of the ZnT-3 mRNA. This certainly is the case with some zinc-containing neurons, such as the granule neurons of the dentate gyrus, which are labeled by the ZnT-3 immunohistochemistry (Palmiter et al. 1996, Wenzel et al. 1997). One assumes that it would be true for all of the zinc-containing neurons, but as mentioned, some caution might be advisable until the performance of a direct, double-staining comparison of cell-by-cell labeling with the mRNA for ZnT-3 and retrogradely transported ZnSe.

Anatomy of the zinc-containing circuitry

Zinc-containing somata are located almost exclusively in the cerebral cortex (including allocortex) and in the amygdalar nuclei (Casanovas-Aguilar et al. 1998, Christensen and Frederickson 1998, Hill and Frederickson 1988, Perez-Clausell et al. 1989, Slomianka et al. 1990). Efferent zinc-containing fibers from these regions are in turn directed almost exclusively to (*1*) cerebral cortex and amygdala (*2*) striatum or (3) "limbic" targets such as septum, nucleus of the diagonal band and medial hypothalamus (Christensen and Frederickson 1998, Howell et al. 1991, Long et al. 1995, Mandava et al. 1993, Perez-Clausell et al. 1989) (Figs. 1, 2). This is in stark contrast to the other chemospecific systems, such as cholinergic, serotonergic, adrenergic and dopaminergic, all of which ascend from subcortical origins to terminate diffusely throughout the hemispheres. One might say that the zinc-containing neurons are an exclusive "output voice" of the cerebral cortex and amygdala.

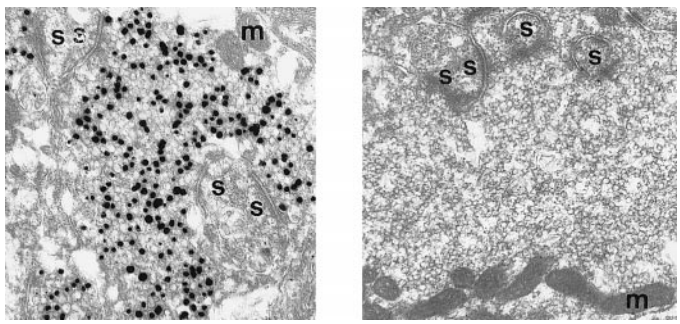

FIGURE 6 Electron microscopic results showing that ZnT-3 knockout mice have no zinc in their gluzinergic boutons. Modified Timm's method shows zinc in a mossy bouton (*left panel*), invaginated by multiple spines with asymmetrical specializations (s). A similar mossy bouton from a ZnT-3 knockout mouse (*right panel*) shows no zinc staining. (Courtesy T. Cole). M = mitochondrion.

It further appears that all zinc-containing neurons are glutamatergic, with the zinc-containing neurons composing a select subset of the broader set of all glutamatergic neurons. The evidence for the zinc-glutamate link is of several types. On a macroscopic level, it is clear that none of the major aminergic or cholinergic systems have zinc-containing somata in the nuclear groups (i.e., basal nucleus of Mynert, Raphe nucleus or substantia nigra) from which they originate (Frederickson 1989). It is also clear that zinc-containing boutons are conspicuously absent in regions (e.g., the pyramidal stratum of the hippocampal formation) where the terminals of GABAergic neurons are densely concentrated (Perez-Clausell and Danscher 1985).

On a finer level of analysis, colocalization studies have established that boutons sequestering γ-aminobutyric acid (GABA) do not sequester zinc (Beaulieu et al. 1992), whereas boutons that are immunoreactive for glutamate do include a high proportion of zinc-containing boutons (Beaulieu et al. 1992, Wenzel et al. 1997). This latter result is further supported by the dendromorphology and location of the zinc-containing neuronal somata that is seen after retrograde labeling of the zinc-containing somata. Pyramidal neurons are the rule in the neocortex and hippocampal formation; horizontally oriented interneurons in nonpyramidal strata are essentially never labeled as zinc-containing somata (Howell and Frederickson 1989, Long et al. 1995, 1997, Slomianka et al. 1990). In view of the strength of the evidence, it may be heuristically useful to identify these zinc-containing glutamatergic neurons as "gluzinergic."

When viewed as a cerebral network, the zinc-containing system appears to have several key anatomical "nodes." One such node is the perirhinal cortex. Zinc-containing neurons in the perirhinal cortex project widely throughout the neocortex and allocortex and to the septum, as well (Howell et al. 1991, Long et al. 1995, Mandava et al. 1993) (Fig. 2). The perirhinal cortex arguably sends zinc-containing fibers to more target regions that any other single efferent zinc-containing system. The perirhinal cortex is also heavily innervated by zinc-containing boutons, with the entorhinal-perirhinal boundary always crisp and stark in zinc histochemistry Fig. 4.

A second "node" in the zinc-containing system is the small blade of pyramidal neurons that lies in the confluence of the subiculum and the hippocampal CA1 pryamidal field. The "prosubicular" neurons (Long et al. 1995, Mandava et al. 1993) are apparently all zinc containing. Like the perirhinal neurons, they project to a large number of targets, including septum, subiculum and several hippocampal fields. The neurons of the subiculum proper, however, are entirely non–zinc containing (Long et al. 1995, 1997).

The amygdalar complex and the hippocampal formation (including the subiculum) are also major points of convergence within the zinc-containing neuronal network. All amygdalar nuclei receive some zinc-containing input, and most of the nuclei also send zinc-containing efferents to both local and remote targets (Christensen and Frederickson 1998, Christensen and Geneser 1995, Howell et al. 1991). Amygdalofugal zinc-containing systems project broadly to the bed nucleus of stria terminalis, pyriform cortex, striatum and periamygdalar cortices. Like the amygdalar complex, the hippocampal formation is another intriguing case. It appears to have four sets of exclusively zinc-containing neurons (dentate granule neurons, CA3 pyramidal neurons, CA1 pyramidal neurons and prosubicular neurons) arranged in a serial circuit that terminates in part within a fifth population (subicular pyramids) that are entirely non—zinc containing (Frederickson and Danscher, 1990, Long et al. 1995, 1997). Another allocortical region with a prominent population of exclusively zinc-containing neurons is the pyriform cortex: all of the pyriform output pyramids are zinc containing (Christensen et al. 1992, Frederickson and Danscher 1990).

One organizational feature of the zinc-containing neuroarchitecture that stands out is that zinc-containing fiber systems tend to be associational, rather that direct, long-pathway systems. In particular, zinc-containing neurons are generally not found in pathways that run either to or from thalamic, subthalamic, brain stem or spinal structures. Instead, the zinc-containing systems are generally corticocortical, corticolimbic or limbic-cortical. Several examples of this pattern may be noted. In primary sensory cortex, for example, zinc-containing boutons are conspicuously absent in layer IV, where the primary thalamocortical afferents terminate (Casanovas-Aguilar et al. 1995, Dyck et al. 1993). In fact, the boundaries between thalamocortical input columns have some scattered zinc-containing inputs even in layer IV, giving cortex such as the striate cortex and the barrel fields a conspicuous, columnar appearance in tissue stained for zinc-containing boutons (Dyck et al. 1993). The laminar segregation of zinc-containing and non–zinc-containing fibers is especially interesting in view of the fact that both sets of afferent fibers are glutamatergic.

The same pattern of exclusion of zinc-containing fibers is found in the innervation of the striatum: corticostriatal fibers that have collateral axons destined for the brain stem and cord (i.e., from Betz cells) are not zinc containing, nor are those originating in brain stem or subthalamus (Howell et al. 1989). In contrast, the corticostriatal fibers that arise from the small cortical pyramids of layers II–IV and deep layer VI are zinc containing (Howell et al. 1989).

On the corticofugal, efferent side, it is generally true that axons destined for thalamic, brain stem or spinal targets are never zinc containing, whereas those destined for cortical or "limbic" targets (amygdalar, septal or hypothalamic) are in part zinc containing. This is illustrated in the motor cortex, where none of the Betz cells are zinc containing, and more broadly throughout the neocortex, where the larger pyramidal neurons of layer V are virtually always non–zinc containing (Casanovas-Aguilar et al. 1995, Slomianka et al. 1990).

The same pattern of segregation can be seen in the hippocampal formation, where the neurons giving rise to purely

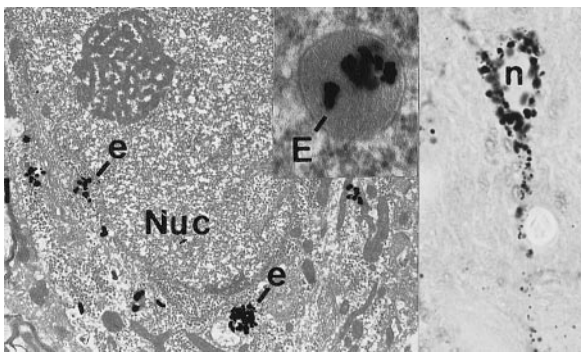

FIGURE 7 Staining of zinc-containing (gluzinergic) soma by retrograde transport. At 24 h after labeling of zinc in boutons by intracranial Se infusion, one sees vesicles or endosomes richly labeled and stained in the perikaryon of the cell. Silver staining of the ZnSe shows clear endosomal localization with use of the light microscope (*right*) and the electron microscope (*left*). Localization within a membrane-enclosed endosome is evident "e" in left panel; "E" in inset. Magnification of inset is ≈100,000×; light microscope magnification is ≈800×. Such endosomal localization is never seen in normal, undamaged neurons (n, Nuc: nucleus).

intrinsic fibers (granule neurons, CA1 neurons) are all zinc containing (Haug 1967, Wensel et al. 1997) and the neurons projecting to other cortical and limbic targets are zinc containing (CA3 neurons; Long et al. 1997), but the neurons that have extensive basal forebrain and brain stem projections (subicular neurons) are entirely of the non–zinc-containing variety (Long et al. 1995, 1997). The pyriform cortex illustrates the same principle in yet another way: The principal pyramidal neurons of the pyriform cortex project exclusively to telencephalic targets (mostly cerebrocortical), and all of those pyramidal neurons are zinc containing (Frederickson and Danscher, 1990).

Why zinc is needed in cerebrocortical "associational" glutamatergic pathways but not in the "long axon" glutamatergic systems is enigmatic. Jacobson (1991) pointed out that neurons with large cell bodies and long axon (type I) are generally precocious and genetically "hard wired," whereas short axon small neurons (type II) are generally later-born and more plastic in both number and connections. One supposition is that vesicular zinc is somehow enabling to the plasticity of synaptic connections of the type II neurons.

To complete a discussion of the zinc-containing anatomy, it must be noted that there are zinc-containing boutons in the dorsal cochlear nucleus that appear to arise from intrinsic neurons, probably cochlear granule neurons (Frederickson et al. 1989). These zinc-containing granule neurons are embryological sisters of the cerebellar granule neurons. Both innervate their respective principal cells with parallel fibers (Frederickson et al. 1989) and both are glutamatergic, yet one group is zinc containing (cochlear) and the other is non zinc containing. Competitive-binding cDNA expression comparisons of these two types of neurons would be fascinating.

Beyond the cochlear nucleus, there are "Timm's-positive" boutons scattered through the thalamus, brain stem and spinal cord (Haug, 1975, Schroder et al. 1978). There also are submammalian species with Timm's-positive boutons (Holm et al. 1988). Whether these boutons contain zinc (which seems likely) or some other metal has not been established to our knowledge. However, the best guess is that the metal is zinc, and how these systems fit into the overall framework of gluzinergic neuronal function can only be imagined.

Molecular biology of zinc-containing neurons

Life cycle of the zinc-containing synaptic vesicle: storage, release and reuptake. *Storage.* Zinc-containing neurons must accumulate, sequester and release zinc from their presynaptic vesicles. The zinc transporter ZnT-3, which is expressed exclusively in the brain (Palmiter et al. 1996), apparently serves to pump zinc into the vesicles. Knockout mice lacking the ZnT-3 gene have no histochemically reactive zinc in their vesicles (T. B. Cole, personal communication) (Fig. 6). Moreover, mutant mice lacking a key protein involved in vesicle cargo selection (mocha mice, lacking AP-3 d) are unable to insert the ZnT-3 protein in the vesicles destined for the presynaptic boutons, indicating that AP-3 is required to sort the ZnT-3 protein into the neurosecretory vesicles. Like the ZnT-3 knockouts, the AP-3–deficient mutants also fail to sequester zinc in the boutons of their zinc-containing neurons (Kantheti et al. 1998).

Another key protein group involved in zinc traffic is the metallothioneins (MT) MT-I, MT-II and MT-III (Aschner et al. 1997, Palmiter et al. 1996). The MT are metal-sequestering proteins. MT-3 is expressed only in the brain and is preferentially located in regions (e.g., the hippocampal formation) that have a high density of zinc-containing neurons (Masters et al. 1994). Mice lacking the MT-3 gene have normal zinc in their zinc-containing boutons (Ericksen et al. 1997), indicating that MT-3 is not an obligatory carrier in the process of getting zinc into neurosecretory vesicles. Moreover, because MT-3 is abundant in neurons that are not zinc-containing neurons (e.g., the pyramidal neurons of the subiculum and principal cells in the cerebellar deep nuclei), it appears that MT-3 is not uniquely associated with zinc transport in the zinc-containing neuron. The fact that MT-3 knockouts have no loss of vesicular zinc (Ericksen et al. 1997) bears this out.

Both ZnT-1 and ZnT-4 transport zinc out of cells, across the plasma membrane (McMahon and Cousins 1998). Although these pumps would not be required within zinc-containing neurons per se, they may be essential (along with MT-3) for neurons that receive zinc-containing synaptic input (i.e., zinoceptive neurons), as discussed later.

Zinc-containing neurons must concentrate zinc intracellularly and then concentrate the zinc further within the secretory vesicles that are destined for the boutons. There is essentially unanimous agreement (Beaulieu et al. 1992, Haug 1967, Palmiter et al. 1996, Perez-Clausell and Danscher 1985) that the zinc is exclusively located in the small clear round vesicles (of glutamatergic neurons). How much zinc is actually in those vesicles is undetermined, but it can be roughly estimated from prior data that show the amount of zinc in the neuropil of one zinc-containing pathway (hippocampal mossy-fiber pathway) ≈350 μmol/L in the wet tissue (Frederickson et al. 1982, calculations in Frederickson 1989, Wensink et al. 1987). To our knowledge, the volume fraction of vesicles in the mossy-fiber neuropil has never been calculated, but there is no doubt that it is among the highest in the brain. (This is, after all, why the mossy-fiber stratum was originally called "stratum lucidum.") An order-of-magnitude guess would be that the vesicles account for 25% of the tissue volume. This implies that vesicles contain concentrations of zinc up to four times 350 μmol/L: 1.4 mmol/L.

The transporter ZnT-3 that decorates the membranes of small clear round vesicles (Wenzel et al. 1997) is presumably responsible for maintaining the high intravesicular zinc content. It is not possible to stain zinc in vesicles in the soma or Golgi apparatus of zinc-containing neurons, even after treatment with colchicine (unpublished observation). It is, however, possible to stain the vesicles in transit in the axons (e.g., Frederickson and Danscher 1990) and, of course, at their destination in the presynaptic boutons. This suggests that the zinc is steadily accumulated in the newly formed vesicles after they leave the Golgi apparatus and while they are in transit to the boutons. One possible explanation for the high variability of zinc staining among individual vesicles (Perez-Clausell and Danscher, 1985) is that denser staining simply indicates more "mature" vesicles that have taken up more zinc.

The availability of zinc in the cytosol for transport into vesicles by ZnT-3 is governed by (*1*) passive channel-mediated flux into or out of the cell, (*2*) active uptake into the cell, (*3*) export out of the cell and (*4*) intracellular zinc–binding ligands. Passive flux through the neuronal membrane occurs when the extracellular level of free zinc is high, as happens when either exogenous zinc is added to a culture medium or when the zinc-containing boutons in the tissue "dump" their vesicular zinc in massive excess, as occurs during excitotoxic events. Weiss et al. (1995 characterized three zinc-permeable channels that can be demonstrated in dissociated neuron cultures: (1) a zinc- and calcium-permeable voltage-controlled channel, (2) an *N*-methyl-D-aspartate (NMDA)-gated channel and (3) an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA)/KA-gated channel. All three of those channels

allow zinc influx when exogenous zinc is added to the medium in amounts (i.e., < 30 μmol/L) that are not cytotoxic.

The active pumping of zinc into neurons or isolated synaptosomes has been explored by several groups who have identified energy-dependent transport with apparent K_m values that range from 0.2 to 10 μmol/L (Colvin 1998, Colvin et al. 2000, Howell et al. 1984, Wensink et al. 1988). Similar results have been obtained with other cell types (Chang et al. 1998, Gisbert-Gonzalez & Torres-Molina 1996, Eide 1997). However, as Wensink et al. (1988) pointed out, there is almost certainly substantial error involved in experiments that simply add micromolar amounts of Zn^{2+} salt to a tissue system. One source of error is the well-established fact that even a Zn^{2+} concentration of 100 μmol/L or so kills cells rather quickly (Yokohama et al. 1986). The other source is that zinc-binding ligands in the extracellular medium of a cell preparation (albumin, histidine, glutathione, and so on) will avidly bind Zn^{2+}, reducing the true concentration of free zinc ion to far below the amount of added zinc ion (total zinc) (Wensink et al. 1988). The use of a "zinc buffer" at a maximum of 10 μmol/L or so solves these problems.

Once free zinc enters a cell, sequestering by MT, calmodulin, S100, tubulin and other ligands is inevitable; the resting level of intracellular zinc in neurons is probably in the range of 50–500 pmol/L (Cheng and Reynolds 1998). This implies that the ZnT-3 pump can concentrate zinc in vesicles against a gradient of eight orders of magnitude.

It is worth noting that the concentration of zinc in presynaptic vesicles of neurons is part of a broad and constant pattern in zinc metabolism wherein certain types of secretory cells concentrate large amounts of weakly coordinated zinc within their secretory granules. We have referred to this as "storage granule zinc" in the past. Examples abound. Storage granule zinc is found the insulin granules of β-cells (Dodson and Steiner 1998), in salivary gland cells (Frederickson et al. 1987), in a variety of leukocyte secretory granules (Danscher, G. & Frederickson, C. J., unpublished results) and in pituitary secretory cells (Thorlacius-Ussing et al. 1985). Zinc (and other exogenous metals) will also be routinely found in perikaryal endosomes whenever cells or tissue are challenged with an excess of metals. This is found when many cell types are exposed to toxic metals (Rungby et al. 1990) and when tissues, such as gill tissue, are exposed to excess metal ions in the water (e.g., Baatrup and Danscher 1987).

Recent evidence suggests that the tendency of cells to sequester metal in vesicles is also triggered when they are maintained in dissociated cell culture. Both Zalewski (2000) and O'Halloran (2000) have adduced evidence that endosomal sequestration may occur under these conditions. Because healthy control neurons never show any perikaryal endosomal zinc deposits in vivo (Howell and Frederickson, 1989, Perez-Clausell and Danscher 1985, 1986), the factors that induce this event in cultured cells or cells challenged with excess metal will be intriguing to understand.

Release. The release of zinc from the presynaptic vesicles of zinc-containing neurons is depolarization and calcium dependent (Howell et al. 1984) and probably occurs via the mechanism of exocytosis of the small clear round vesicles in which silver staining shows the zinc (Perez-Clausell and Danscher, 1986). Many groups have demonstrated release (Aniksztejn et al. 1987, Assaf and Chung 1984, Sloviter 1985). Release has an apparently high Q_{10}, being virtually completely blocked at room temperature (20–26°C) and vigorous in the range of normal rat body temperature, 37–39°C (Frederickson et al. 1998). This explains why experiments done at or near room temperature basically fail to demonstrate physiological release of zinc (Kay et al. 1995). Furthermore, in the case of conventionally cut brain slices, it turns out that up to half of the bouton zinc is irretrievably lost through the act of cutting the slice (Frederickson et al. 1998). These two problems are major obstacles to the study of zinc release in the typical in vitro slice preparation. They may also explain why zinc chelation typically produces only weak or mixed effects on monosynaptic evoked responses in the in vitro slice preparation when tested below normal rat body temperature (Easley et al. 1993, Frederickson 1989, Xie and Smart 1994).

Essentially nothing is known about the kinetics of zinc release. We previously speculated that zinc release might be sharply nonlinear with frequency of firing, based on fairly scanty evidence (Easley et al. 1993), but there are no real data on this point. One assumes that zinc and glutamate are corereleased, and there certainly are many conditions in which both substances can be shown to have been released, such as after ischemia, seizures and blunt trauma (Frederickson et al. 1988, 1998, Sorensen et al. 1998). Still, the corelease of glutamate and zinc has never been directly demonstrated or characterized, an important oversight in this field.

It has been shown clearly in many laboratories that the total amount of zinc stored in vesicles can be released quickly, with a resulting depletion of the boutons. This was first reported by Sloviter in 1985, and is seen best after excitotoxic insult (seizures, head injury, ischemia) when the loss of stainable zinc from the boutons can be virtually complete within as little as 1 h (Frederickson et al. 1988, Koh et al. 1996, Sorensen et al. 1998, Suh et al. 1995, 1996, Tonder et al. 1990). Where the zinc goes after release is not yet fully resolved (see later). However, in situ labeling of the zinc in an effort to "follow" released zinc has revealed some intriguing complexity of the release process. Thus, precipitating the zinc in situ in the vesicles with sulfide in vivo leads to an almost complete translocation of the ZnS into the cleft or to the presynaptic specialization within 12 h (Perez-Clausell and Danscher 1986), whereas precipitating in situ with selenium leads to some exocytosis but a preferential retrograde transport of the ZnSe-filled vesicles to the perikaryon (Howell and Frederickson 1990) (Fig. 7). Binding zinc in vitro with lipophilic, toluene sulfonamid quinoline (TSQ) derived chelators leads to a relative immobilization of the zinc-quinoline complex in the membranes (Andrews et al. 1995, Kay et al. 1995). In short, once the speciation of the relatively weakly-bound zinc has been perturbed in the vesicles, whatever happens thereafter is variable and nonphysiological.

It is widely assumed that the synaptic zinc that is released from boutons actually enters the extracellular fluid as Zn^{2+}, but in fact, this has never been verified. We have preliminary evidence showing that the zinc released by electrical stimulation can be readily bound by an extracellular fluorescent zinc probe, suggesting that the zinc is indeed released as Zn^{2+} (**Figs. 8**, **9**). The kinetics and biophysics of this release are under investigation with this method.

Reuptake. Where zinc goes in the normal, intact brain after release is also enigmatic. Certainly some can enter neurons that are postsynaptic to gluzinergic boutons ("zincoceptive" neurons), as discussed later. Some is perhaps taken back into the bouton and recycled into vesicles. Certainly uptake into the neuropil, into synaptosomes and into artificial "endosomes" have all been demonstrated (Colvin 1998, Howell and Frederickson 1984, O'Halloran 2000, Wensink et al. 1988). However, it is important to recall that zinc-containing boutons can readily be depleted of their stainable zinc by intense activation in vivo and in vitro (Frederickson et al. 1998). This is quite different from

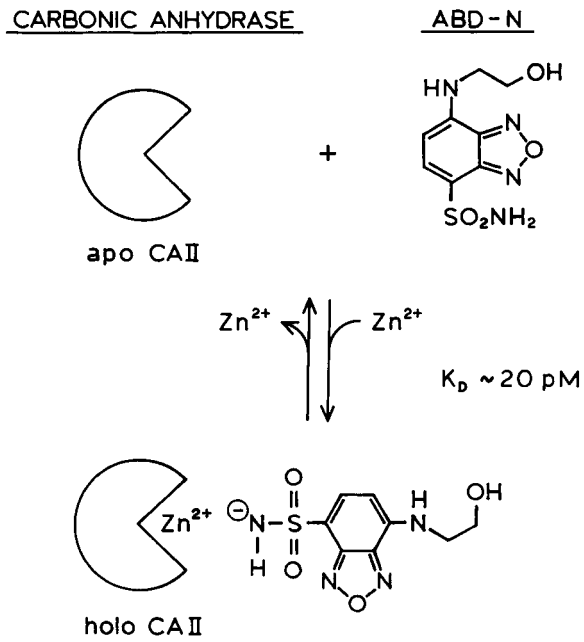

FIGURE 9 Carbonic anhydrase–ABD-N zinc fluorescent probe (EnZin). The apo carbonic anhydrase cannot penetrate cell membranes. Thus, when placed in extracellular fluid, this system detects only extracellular Zn^{2+}.

small molecule transmitters, such as the amines or acetylcholine, which are generally not depleted during intense activation unless reuptake is blocked. Thus, in the zinc-containing bouton, either reuptake of zinc into boutons is quantitatively unimportant or, plausibly, the pathological conditions that lead to zinc "dumping" also lead to inhibition of the putative zinc reuptake system. The latter scenario appears to occur with glutamate, with which both accelerated released and suppressed reuptake occur in some excitotoxic situations (Zhao et al. 1997). The modulation of zinc reuptake is a potentially important therapeutic strategy for neuroprotection against excitotoxicity.

Besides reuptake and penetration into postsynaptic neurons, the third major "sink" for released zinc is the population of zinc-binding ligands in the extracellular fluid, including albumin, glutathione, histidine and others. These are present at low micromolar concentrations and have K_d values for zinc binding in the low nanomolar range (Wensink et al. 1988). Thus, so long as they are not saturated by an excess of zinc, these "scavengers" would be expected to rapidly remove the ionic zinc released from zinc-containing boutons, maintaining the low nanomolar extracellular concentration during normal physiological conditions.

Normal physiological function of synaptic zinc

The colocalization of zinc and glutamate implies that zinc is involved in the function of the glutamatergic synapses. There are three loci at which the zinc might cooperate in glutamatergic function: in the vesicle, in the cleft or in the postsynaptic neuron.

One venerable notion of the role of zinc is that the zinc in the vesicle is important for glutamate storage. In theory, zinc could either increase the storage capacity (by polymerization/precipitation of the glutamate) or slow the release rate of glutamate by imposing the slow kinetics of some Glu-Zn dissociation process. These theories are weakened (although not fatally) by the fact that many groups have found that removal of the endogenous zinc through chelation or precipitation has no demonstrable effect on the single-pulse, monosynaptic evoked response at zinc-containing synapses (Easley et al. 1995, Mitchell and Barnes 1993, Xie et al. 1994).

Concerning the role or roles of zinc in the cleft, it is quite clear that zinc ions are powerful modulators of both ionotropic and metabotropic NMDA and KA receptors for glutamate. Zinc-specific binding sites have been identified (Smart et al. 1994), and the effects of zinc are seen at concentrations in the range of 1–10 μmol/L, a level that would be easily obtained in the immediate microenvironment of a zinc-containing bouton during zinc release (see later).

The functional significance of the modulatory role of zinc is hard to predict, because both up- and down-regulation of receptor function occur at different receptors (Smart et al. 1994), and even at different splice variants of the same receptor (Hollmann et al. 1994). This notwithstanding, most evidence suggests that zinc is predominantly defacilitatory, or anticonvulsant, when whole animal or brain slice systems are tested. Thus, zinc chelation is frankly proconvulsive (Mitchell et al. 1990, Mitchell and Barnes 1993), and knockout mice ($ZnT\text{-}3^{-/-}$) lacking vesicular zinc are especially seizure prone (Wenzel et al. 1997). The intracerebral administration of Zn^{2+} kills neurons (Lees et al. 1998, Yokahama et al. 1986), so any anticonvulsive (or proconvulsive effect) is essentially impossible to assess in the face of the direct damage.

As mentioned earlier, the removal of zinc through chelation or precipitation has little or effect on the monosynaptic evoked response at glutamatergic, zinc-containing synapses. There is some evidence that the synaptic function observed during high frequency driving might be selectively sensitive to zinc depletion (Easley et al. 1995), but this is controversial (Xie et al. 1994). In any event, the discovery of a preparation and paradigm that would reveal a robust and consistent effect of removing the normally present, endogenous zinc from the presynaptic vesicles would be a major advance for the field.

Beyond the glutamatergic synapse, there are dozens of membrane-spanning proteins sensitive to zinc, including receptors, channels, pumps and proteins such as β-amyloid and α-macroglobulin (Frederickson 1989, Smart et al. 1994). Among these, the GABA receptor has received especially active attention and has proved to be sensitive to micromolar amounts of zinc. Although the complete story is complex (Smart et al. 1994), there appears to be some consensus that zinc ions decrease inhibitory drive from the $GABA_A$ receptor in the normal adult, a notion that is hard to fit with the generally proconvulsive effects of zinc depletion noted earlier.

Ultimately, which of the zinc-sensitive proteins in the brain will actually be modulated by zinc will depend on whether zinc ions could ever reach the proteins in question. In this regard, it is useful to consider concentrations. If a single bouton [e.g., 1 μm^3 (10^{-15} L) in volume] had 1 mmol/L average zinc content, it would release $\approx 1\ 10^{-18}$ moles, or $\approx 10^5$ zinc ions. This assumes a massive, complete "dump" of all the zinc of the bouton. Within only a 15-μm radius from the bouton, the ions will be diluted by $1{:}15^3$ ($\approx$1:3000), i.e., from 1 mmol/L to $\approx$300 nmol/L. Because only $\approx$10% of that volume is extracellular fluid, the extracellular concentration could reach $\approx$3 μmol/L, which is near threshold for most zinc-sensitive receptors (Smart et al. 1994). However, the binding of the Zn^{2+} to extracellular ligands such as albumin and glutathione alone (both present at $\approx$1 μmol/L in the cerebrospinal fluid and both with K_d values in the nanomolar range; Cousins 1986, Wensink et al. 1988) would rapidly lower that $\approx$3 μmol/L Zn^{2+} to the low nanomolar range.

In short, the "zinc signals" released from gluzinergic terminals may normally travel no more than a few microns before dissipating into the background zinc. Thus, for example, $GABA_A$ receptors studding the membranes of granule neuron somata may never be reached by zinc ions that can only be released from boutons up in the molecular dendritic zone or the subgranular hilar zone of those granule neurons. On the other hand, where GABA receptors are strewn amid gluzinergic boutons, as in the stratum radiatum dendritic zone of the hippocampus (Sperk et al. 1997), synaptically released zinc could potentially modulate GABA function.

The third, and most speculative, of the possible roles of zinc are those that could come into play if zinc ions were to move from the presynaptic bouton into the interior of postsynaptic neurons. This admittedly heretical idea has no direct support, but it is indirectly supported by the evidence that zinc is apparently translocated in just such fashion during excitotoxic brain injury (see later). There are literally dozens of intracellular proteins that are sensitive to zinc ions (Cuajungco and Lees 1997), including those in the cytosol, in mitochondria and, perhaps most intriguingly, in the nucleus. There is evidence that zinc ions in physiologically extreme conditions can modulate the probability of apoptosis (Aiuchi et al. 1998, Cuajungco and Lees 1997). Virtually all DNA-binding proteins are zinc finger proteins (Choo and Klug 1997). The possibility that zinc signals might play on that family of gene-regulatory proteins, guiding gene expression in vital ways, makes zinc potentially the most powerful of all synaptic messenger substances. Direct testing of whether synaptically released zinc enters postsynaptic neurons is critically needed.

Synaptically released zinc and pathology. *Excitotoxicity.* Synaptically released zinc is directly implicated in the phenomenon of excitotoxicity, in which brain tissue deprived of oxygen, glucose or both begins to release massive amounts of excitatory amino acid transmitter (e.g., glutamate), neurons are tonically depolarized and cell death, both immediate and delayed, ensues.

The first evidence of the role of zinc in excitotoxic neuron death was obtained in rats that had experienced kainic acid (KA)-induced seizures (Frederickson et al. 1988) (**Fig. 12**), a finding that capitalized on earlier evidence of the toxicity of zinc to cultured neurons (Yokohoma et al. 1986). That seizure result was soon replicated (Frederickson et al. 1989) and extended to the ischemia model (Tonder et al. 1990), with the basic finding that zinc (*1*) disappeared from presynaptic boutons and (*2*) appeared selectively in dying postsynaptic neurons during excitotoxic events. The notion that such "translocation" (Frederickson et al. 1989) of zinc from presynaptic boutons to postsynaptic neurons could kill the target neurons was finally given a definitive and direct test in 1996 by Koh et al. (1996), who marshalled quite elegant and convincing data on that point.

The depletion of zinc from the boutons after excitotoxic insults has been verified for ischemia (Sorensen et al. 1998, Suh et al. 1995), seizures (Frederickson et al. 1988, Sloviter 1985, Suh et al. 1996) and traumatic head injury (Long et al. 1998) (**Fig. 11**). Moreover, several groups have repeated and extended the basic zinc-chelation/neuroprotection phenomenon, which is as follows. In paradigms in which neuron death is produced by ischemia, seizures, or traumatic head injury, the simple expedient of chelating extracellular zinc just before (and during) the insult will protect neurons from the excitotoxic injury, yielding up to 85% neuron sparing compared with the unprotected control situation (Choi and Koh 1998, Koh et al. 1996, Long et al. 1998, Suh et al. 1995, 1996).

Compounds that are membrane permeable and can act on zinc-dependent or zinc-containing metalloenzymes do not protect neurons, instead producing either mixed, contradictory or weak effects in various excitotoxic paradigms. This includes a variety of chelators, such as TPEN, DEDTC, dithizone and membrane-permeable derivatives of TSQ (Lees et al. 1998, Mitchell and Barnes 1993). The failure of membrane-permeable compounds to afford neuroprotection is presumably due to the fact that the inactivation of essential zinc-dependent biochemistry inside neurons is directly cytotoxic, even cytolethal (McCabe et al. 1993, Zalewski et al. 1994). Likewise, an extracellular chelator that cannot reduce extracellular Zn^{2+} (Zn-EDTA) has no protective effect, as one would expect (Koh et al. 1996, Long et al. 1998, Suh et al. 1995, 1996).

Weiss and coworkers have shown that zinc ingress into neurons during excitotoxic conditions occurs via three channels: an NMDA-gated channel, a voltage-dependent channel and an AMPA/KA-gated channel (Weiss et al. 1993, Yin and Weiss 1995, Yin et al. 1998). The last of these is apparently expressed only in select neurons (which are thus selectively vulnerable) and can carry a much higher zinc "current" that the others. Thus, cells with the KA/AMPA channel are most at risk for zinc-mediated neuron death (Yin et al. 1998).

The details of zinc-induced neuron death are only now emerging, but one mechanism appears to be mitochondrial poisoning, because the ingress of zinc produces a correlated rise in reactive oxygen species (Carriedo et al. 1998). Another line of evidence has indicated that zinc influx can trigger apoptosis in target neurons (Manev et al. 1997, Telford and Fraker 1997). On the other hand, it is also true that zinc deficiency can trigger apoptosis in some cells and model systems (Ahn et

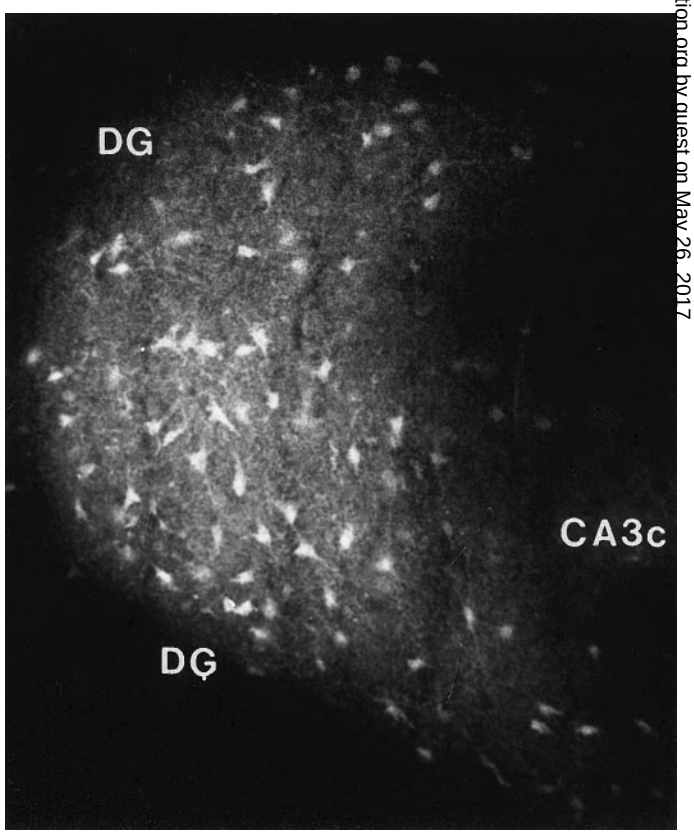

FIGURE 12 Zinc translocation after KA-induced seizures. Hilus of the hippocampal dentate gyrus (DG) after TSQ staining, after 6 h of status epilepticus induced by KA. Although presynaptic zinc fluorescence is reduced (note total loss in hippocampal CA3c area), many hilar cell bodies exhibit strong TSQ staining. This staining for zinc in neuronal perikarya indicates zinc-induced damage.

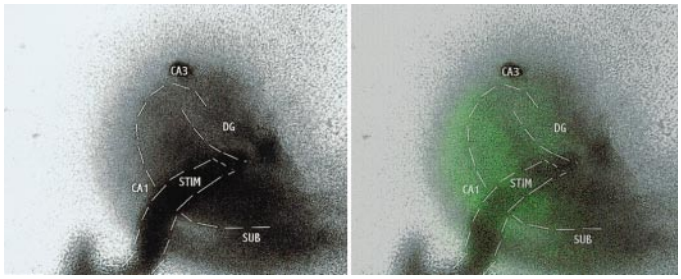

FIGURE 8 Stimulation-induced zinc release from neuropil of hippocampal culture. *Left panel*: An organotypic culture of rat hippocampus (31 d old) is shown in pseudophase on the left. An electrode (STIM) is in position on the dentate gyrus (DG), and CA3, CA1 and some subiculum (SUB) are intact in situ after a 20-s train of stimulation (100 MHz; 0.5 ms; 200 μA). Zinc released into the extracellular fluid is chelated by the carbonic anhydrase ABD-N extracellular zinc probe, yielding a bright green fluorescence in the fluid over the neuropil.

al. 1998), with zinc administration providing a remedial decrease in the apoptosis (McCabe et al. 1993, Zalewski et al. 1994). Thus, the exact mechanism of zinc-induced neuron death remains unresolved.

In addition to the harm done by zinc entering the neurons, it should be mentioned that the high Zn^{2+} concentrations occurring extracellularly could also exacerbate the excitotoxic

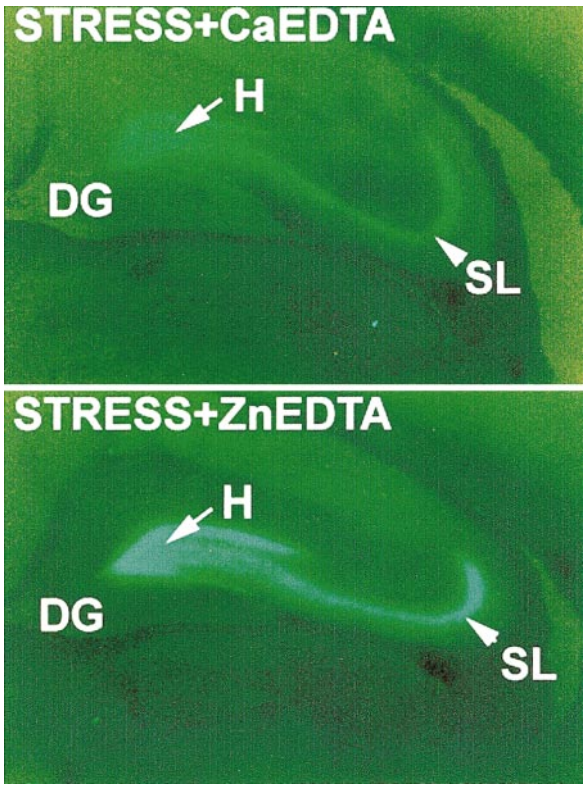

FIGURE 10 Zinc depletion from hippocampal mossy fiber after restraint stress. *Upper panel*: TSQ fluorescence staining 4 h after restraint stress with the administration of non-zinc chelator (Zn-EDTA). Note the normal fluorescent staining of zinc-containing boutons. *Lower panel*: Same stress but any synaptically released zinc was now chelated by the extracellular chelator Ca-EDTA. Note the profound loss of zinc. Paired rats kept in their home cages for the same 4 h showed no difference in zinc loss. H = hilus, DG = dentate syrus, SL = stratum lucidum.

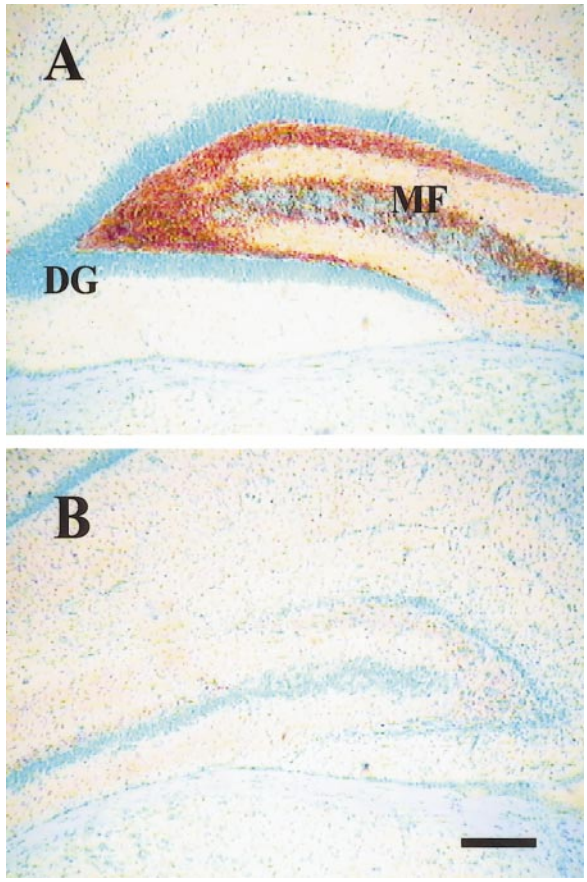

FIGURE 11 Zinc depletion from hippocampal mossy fiber after brain trauma. At 24 h after impact damage to the exposed dura of the dorsal neocortex, Timm-Danscher staining shows zinc loss in the subjacent hippocampus [especially mossy fibers (MF)] ipsilateral to impact (*B*) but little loss contralateral (*A*). DG, dentate gyrus. Bar = 100 μm.

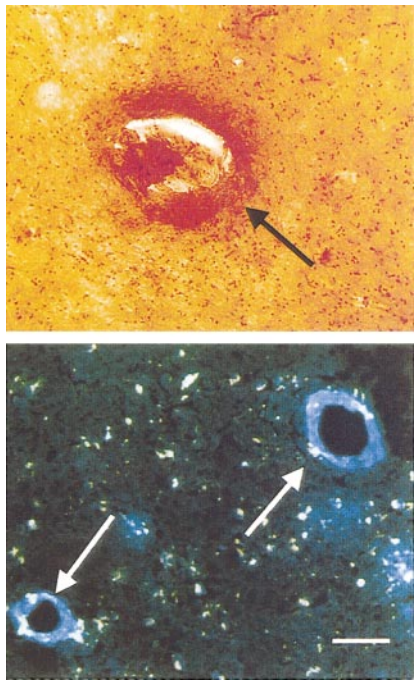

FIGURE 13 Zinc staining in small vessel walls of hippocampus of patient with Alzheimer's disease. *Upper panel*: AMG (Danscher, 1996) staining of typical angiopathy surrounding a small vessel (*arrow*). *Lower panel*: Zinc staining of the angiopathic rings (*arrows*) is shown by TSQ. Scale bar = 100 μm.

cascade. To be specific, elevated extracellular Zn^{2+} can (*1*) impede the reuptake of glutamate (Vendenburg et al. 1998), (*2*) increase membrane depolarization through the up-regulation of KA/AMPA receptors (Peters et al. 1987) and some splice variants of NMDA receptors (Hollmann et al. 1994) and (*3*) contribute to Ca^{2+} dysregulation via effects on Ca^{2+} pumps (Colvin 1998).

Alzheimer's disease. An excellent article on zinc and Alzheimer's disease is given by Huang et al. (2000) and shows the intricate role of zinc (potentially synaptically released zinc) in the etiology and symptomology of that disease. The general notion emerging is that synaptically released zinc can interact with Aβ 1-42 protein, causing the latter to precipitate into plaques and perivascular angiopathy. The major points of evidence are that (*1*) plaques form preferentially in brain regions densely innervated by gluzinergic fibers; (*2*) micromolar amounts of Zn^{2+} precipitate Ab, potentially causing the formation of Ab-rich dense-core plaques in the brains of patients with Alzheimer's disease; (*3*) zinc chelation can solubilize plaque material from brain homogenate; (*4*) plaques in brain tissue obtained at autopsy from patients with Alzheimer's disease are enriched with as much as 1 mmol/L zinc (Lovell et al. 1998); and (*5*) both the tissue distribution of zinc and the histochemical staining for zinc have been reported to be disturbed in Alzheimer's disease (Constantinidis 1990, Danscher et al. 1997) (**Fig. 13**). This last histochemical observation has recently been confirmed and extended in our laboratory in data showing that the dense-core plaques and the perivascular, angiopathic amyloid deposits are rich in histochemically reactive zinc (Suh et al. 1998) (Fig. 13). Whether zinc-induced precipitation of the Aβ into plaques is ultimately neuroprotective or neurodestructive is not fully resolved.

Stress. Hippocampal neurons are among the most sensitive to stress-induced neuron death (Stein-Behrens et al. 1994), and there is one report suggesting that acute immobilization stress can cause some release of zinc from the hippocampus (Itoh et al. 1993) (Fig. 12). We have begun to explore a possible role of stress and the stress hormones in the mobilization and release of zinc from the boutons of gluzinergic neurons, with some intriguing preliminary findings. The first is that the adrenal glucorticoid appears to modulate the level of zinc that is sequestered in the boutons of the gluzinergic neurons. Thus, surgical adrenalectomy causes a drop in the level of bouton zinc in some hippocampal neuropil within as little as 10 d after surgery. This is well before the loss of granule neurons is pronounced and occurs in the absence of a measurable loss of mossy-fiber bouton zinc (Suh et al. 1997). One plausible mechanism linking the glucocorticoid driving of zinc levels would be that glucocorticoid signals increase the rate of synthesis of peptides or proteins (such as nerve growth factor) that might be colocalized with zinc in the presynaptic vesicles.

More recently, we have begun exploring the effects of stress on the release of zinc from gluzinergic boutons. The preliminary finding is that rats subjected to immobilization stress for 4 h show a dramatic loss of bouton zinc compared with rats that are simply left in the home cage (Fig. 12). One especially interesting note about this preliminary result is that we have not seen it in rats that were not pretreated with an extracellular zinc chelator (Ca-EDTA). This implies that the stress actually increases bouton turnover of zinc, a result that will be missed unless the reuptake process is impeded by extracellular chelation of the zinc before reuptake. The implications of a possible stress modulation of zinc storage and zinc release are far reaching.

Conclusions

From its former marginal status as a "trace element," zinc has risen to assume a position along side calcium, potassium and sodium as a key modulator of neuronal excitability. The "gluzinergic" synapse is perhaps the single most abundant synapse type in the mammalian cerebral cortex, giving zinc a privileged role in cortical communication. The role of zinc remains to be discovered. However, the fact that cytolethal amounts of zinc are sequestered in those cerebrocortical boutons implies that regardless of the role of zinc, it is sufficiently vital to outweigh the risk of harboring cell-killing quantities of zinc in the boutons. Discovery of the normal, physiological role of vesicular zinc in the mammalian cerebrocortex remains an exciting challenge for neurobiologists.

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