THE NEUROBIOLOGY OF ZINC IN HEALTH AND DISEASE

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Abstract | The use of zinc in medicinal skin cream was mentioned in Egyptian papyri from 2000 BC (for example, the Smith Papyrus[1]), and zinc has apparently been used fairly steadily throughout Roman[2] and modern times (for example, as the American lotion named for its zinc ore, 'Calamine'). It is, therefore, somewhat ironic that zinc is a relatively late addition to the pantheon of signal ions in biology and medicine. However, the number of biological functions, health implications and pharmacological targets that are emerging for zinc indicate that it might turn out to be 'the calcium of the twenty-first century'.

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To nutritionists, zinc is an essential micronutrient[3]; to biochemists, it is a component of enzymes and other proteins[4]; whereas to environmentalists and marine biologists, free zinc in water is a toxic pollutant[5] (BOX 1). To neuroscientists, zinc is not only a micronutrient and a component of proteins, but is also an ionic signal. Zn^{2+} moves through gated membrane channels[6,7] and among various organelles and storage depots within cells[8,9], modulating protein function by binding to and detaching from zinc-dependent proteins throughout the cell[9–11]. Like calcium, excess free zinc in body tissues is toxic[12].

Zn^{2+} is selectively stored in, and released from, the presynaptic vesicles of a specific type of neuron, which is found chiefly in the mammalian cerebral cortex (FIG. 1). These zinc-releasing neurons also release glutamate, and the term 'gluzinergic' has, therefore, been proposed to describe them[13,14]. Most glutamate- and zinc-releasing neurons have their cell bodies in either the cerebral cortex or the limbic structures (amygdala and septum) of the forebrain[15]. Therefore, the glutamate- and zinc-releasing neuronal system comprises a vast cortical–limbic associational network that unites limbic and cerebrocortical functions.

In the fifty years since zinc's signalling role was first discovered[16], a broad outline of the function of glutamate- and zinc-releasing neurons has emerged (FIG. 2). Zinc seems to modulate the overall excitability of the brain through its effects on glutamate, and probably γ-aminobutyric acid (GABA), receptors[17], and is also thought to be important in synaptic plasticity[18,19].

Here, we describe the biology of glutamate- and zinc-releasing neurons and review the current evidence for the normal function of these neurons and their synaptic zinc signals. We also review findings that implicate zinc signals in the pathophysiology of acute brain damage and degenerative brain diseases.

Discovery of glutamate–zinc neurons

Focal deposits of 'free' or 'exchangeable' zinc were first found in the brain by Maske[16], who used a histochemical method that could detect only the exchangeable zinc. Although he was primarily interested in zinc-secreting pancreatic cells, Maske also looked at the brains of his test animals, where he found a conspicuous, bright red band of zinc–dithizonate staining.

We now know that the band was comprised of hippocampal mossy fibre axons, the giant terminals of which are rich in exchangeable zinc. Moreover, we also now know that the mossy fibres are just one example of many intrinsic, cerebrocortical pathways, the axons of which sequester zinc and glutamate in their synaptic vesicles. Although glutamatergic, the long axon pathways that project into the cerebral cortex and those that project corticopedally to subcortical targets have no vesicular zinc[20–23,24]. Likewise, glutamatergic pathways that originate outside the cerebral cortex and limbic nuclei contain only token amounts of stainable

Box 1 | **Zinc signals outside the brain**

Historically, neurotransmitters and neuromodulators were often identified and characterized in tissues or organs other than the brain, then tracked into the brain and linked to behaviour. For example, acetylcholine was first identified in the heart[240], adrenaline in the vasculature, GABA (γ-aminobutyric acid) in crayfish muscle[241] and peptides in the gut[242]. Research on zinc signalling follows this same historical pattern, in that the first zinc-secreting cells to be characterized were the insulin- (and zinc-) secreting cells of the pancreas and the fluid- (and zinc-) secreting cells of the venom–salivary gland in snakes[243].

Today, there is broad awareness of zinc signalling throughout the body, with a dozen or more individual zinc-secreting cells types known. These zinc-secreting cells include the submandibular salivary gland[244] (modified to a venom gland in snakes), the pancreatic β-cells[245] and pancreatic exocrine cells[245], the prostate epithelial cells[246], Paneth cells in the intestine[247], mast cells[248], granulocytes (three types)[249,250], pituitary cells (four types[251]) and CNS neurons (three types)[128,252]. Intriguingly, the biological and physiological roles of these myriad somatic zinc signals are still largely unknown and unstudied.

metals[25,26]. By contrast, in some areas of the cerebral cortex, glutamate- and zinc-releasing neurons contribute almost half of all of glutamatergic synapses[27].

Measuring synaptic release of zinc. Three methods have been used to show synaptic zinc release: before-and-after imaging of zinc in the boutons, analytical detection of zinc released into perfusates and, most recently, direct imaging of released zinc using fluorescent extracellular probes.

Haug and colleagues launched the before-and-after studies, and showed that staining of vesicular zinc in mossy fibres vanished within hours of axon transection, even though the ultrastructure of the axon terminals remained intact for several days[28]. Many have replicated Haug's basic result, using stimuli such as 24 h of electrical stimulation[29], 2–4 h of status epilepticus[29–31], excitotoxic injury, such as ischaemia–reperfusion[32], and head trauma[33], all of which dramatically deplete the boutons of zinc.

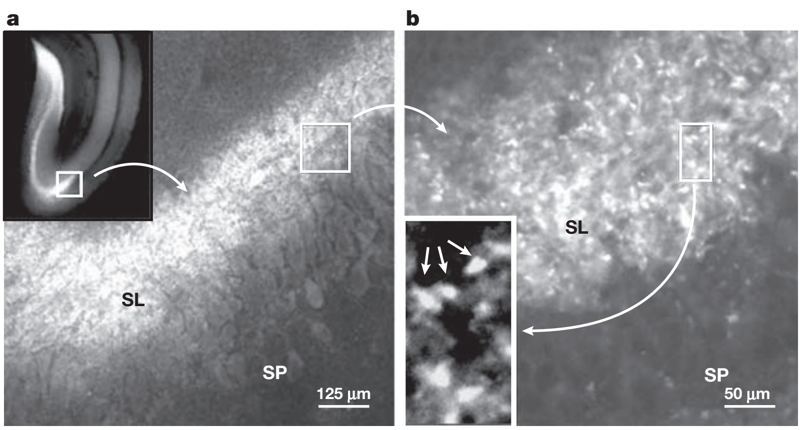

Figure 1 | **Intravital staining of mossy fibres in the rat using the fluorescent probe ZP1.** **a** | Inset is a x4 survey view of the hippocampal formation; the enlargement shows the bright staining in the stratum lucidum (SL) and lack of staining in the stratum pyramidale (SP). **b** | A further enlargement of the SL, showing individual mossy boutons (bright puncta), three of which (arrows) are further magnified in the inset[253].

In a variation of the before-and-after protocol, vesicular zinc is labelled *in situ*, and release of the label is observed. This approach is somewhat problematic, as it is a zinc–label complex that is released, not zinc *per se*. Nevertheless, several groups have shown robust and reliable release of zinc–label complexes from boutons on electrical stimulation[34,35], or over time in the absence of stimulation[36]. Most recently, the release of zinc–*N*-(6-methoxy-8-quinolyl)-p-toluenesulphonamide (TSQ) was elegantly shown on a pulse-by-pulse basis, with each action potential releasing zinc[37]. Several groups have observed calcium-dependent zinc release into perfusates of electrically or chemically stimulated brain tissue *in vivo* and *in vitro*[38–42]. One recent innovation is the use of fluorimetry to distinguish between free zinc and bound zinc in the perfusates of stimulated tissue. Using brain microdialysis, the release of up to 100 nM of free zinc has been observed during excitotoxic stimulation of brain tissue[43].

Direct imaging of synaptic zinc release is the definitive method, and has now been done successfully in four laboratories. In the first study, released zinc was detected by a biosensor, which consisted of a zinc metalloenzyme that lacks zinc (apo-carbonic anhydrase, apoCA) and a fluorescent reporter that shifts emission on binding to holo-carbonic anhydrase (holoCA)[44]. Later work using more direct imaging of tissue slices revealed much faster zinc release (30 ms[45,46]). Three fluorescent probes, each with different kinetics and affinities, have all yielded estimates of the amount of zinc that is released in the 10–30 μM range[43–46]. Of course, such estimates reflect an average amount throughout the tissue, and concentrations in the cleft would presumably be much higher during the brief synaptic release events, whereas concentrations measured with a relatively large dialysis probe would be lower. One caveat that emerges from comparative studies is that the amount of zinc in the vesicles of the mossy axons of young altricial animals (for example, rats) is vanishingly small, and does not mature until ~75 days after birth. This is because the granule neurons are born postnatally, and their mossy axons are correspondingly late to appear and sequester zinc[47,48,49]. So, zinc release from the immature hippocampus is always modest compared with that in the adult hippocampus[50].

Zinc entry into somata and dendrites. Neuronal somata and dendrites are studded with zinc-permeable, gated channels, which include the NMDA (*N*-methyl-D-aspartate) channel, voltage-gated calcium channels and the calcium-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)/kainate channel (Ca^{2+}-A/K). Zinc influx through these channels has been shown using ^{65}Zn tracing[51], nuclear magnetic resonance (NMR) with zinc-specific contrast agents[52] and fluorescent measurements of free intracellular zinc ($[Zn^{2+}]_i$)[30,53–56].

Because presynaptic terminals release zinc, and the postsynaptic somata and dendrites have zinc-permeable channels, it follows that, under favourable

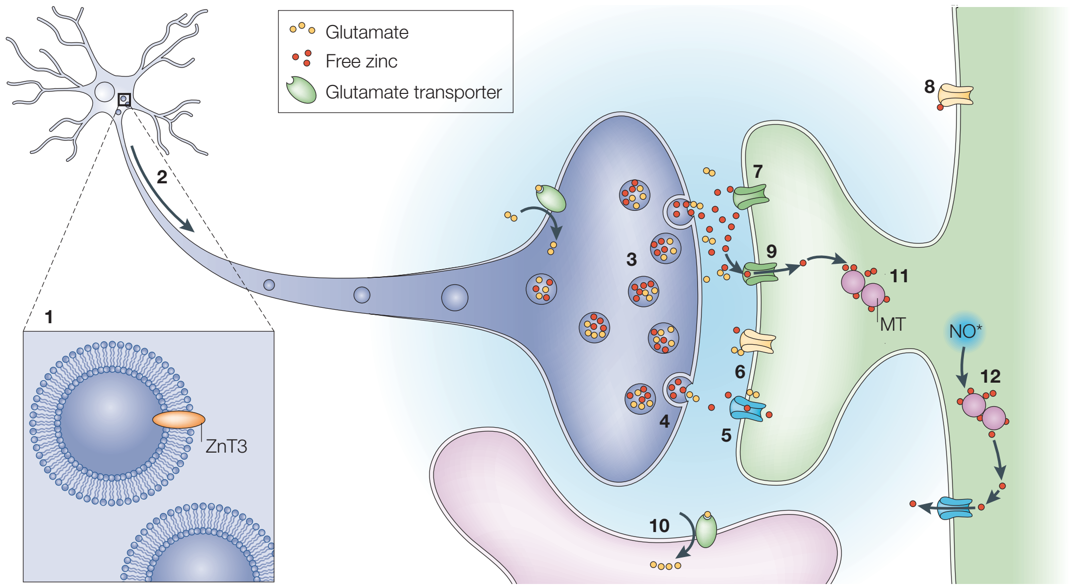

Figure 2 | **Synaptic zinc transport.** Vesicles decorated with the zinc transporter ZnT3 protein are assembled in the Golgi apparatus of glutamate- and zinc-releasing neurons (1) and transported down the axon (2). Once in the presynaptic terminal, the vesicles can be seen to contain both glutamate and free zinc. Calcium- and impulse-dependent exocytosis expels both zinc and glutamate (4), both of which have receptors on the postsynaptic membrane. In some cases, both receptors are components of the same iontophore, as with the GluR and GluM receptors (TABLE 1). Zinc modulates myriad channels, transporters and receptors locally and (perhaps) after diffusing a few tens of micrometres, on neurons and glial cells (6–10). All calcium channels have some zinc permeability (5 and 9), and zinc-permeating postsynaptic neurons are chaperoned by the thionein-metallothionein system (11). Oxidation and nitrosylation of thiols in metallothionein (MT) releases Zn^{2+} from MT (12), possibly leading to 'somatic' release of zinc.

conditions, Zn^{2+} will translocate from inside a presynaptic neuron to inside a postsynaptic neuron. Because both glutamate and depolarization open the zinc-permeable channels[6,57–59], maximum zinc translocation would be expected during intense neuronal activity. There is considerable evidence that such translocation contributes to zinc-induced cell injury in excitotoxicity (see below). There is also evidence that a smaller-volume translocation might occur during physiological synaptic signalling, with the translocated zinc perhaps triggering further signalling cascades in the postsynaptic neuron[18]. However, it is difficult to distinguish those zinc signals that have entered a cell through the plasma membrane from those that have arisen through the mobilization of zinc from intracellular proteins.

Intracellular mobilization and somatic release. In addition to the zinc that is released from presynaptic terminals into the extracellular fluid, there is also a pool of releasable zinc in perikarya. One source of this zinc is the metallothioneins (MTs), from which zinc can be released rapidly by nitrosylation or oxidation of the thiol ligands[60,61].

Thioneins are small proteins (~3000 Da) that contain several cysteine residues that allow them to bind metals, including zinc[62]. They function physiologically by accepting zinc from other zinc-binding ligands, including proteins. Thionein can bind seven zinc atoms through 20 cysteine residues in zinc clusters[63]. Oxidation or nitrosylation of cysteine residues in the zinc cluster results in the release of zinc[64], so these proteins can function as zinc donors to other zinc-binding proteins. Metallated thionein is in equilibrium with the unmetallated (or apo-) thionein[65].

The metallothionein 3 (MT3) isoform is found only in the brain and testes, whereas other isoforms are more widespread[66,67]. In mice that lack MT3, cell injury in hippocampal field CA1 and the thalamus is significantly reduced after brain injury[68], which implies that zinc released from MT3 can contribute to cell injury. By contrast, in hippocampal field CA3, loss of MT3 increases cell death after excitotoxic injury, presumably because the presynaptic release of zinc is so pronounced in CA3 (REF. 46) that the postsynaptic MT3 functions more as a zinc sink than a zinc source.

Given that nitric oxide (NO) can mobilize zinc from proteins (notably MT3), and that neuronal somata fill up with free zinc from this source under excitotoxic conditions, it is possible that zinc could flow directly from the perikaryal cytoplasm into the surrounding extracellular milieu[69]. Zinc-permeable channels[6, 70,71] or transporters[72,73] could mediate this somatic zinc 'release'. This phenomenon has not been shown directly, but images of zinc effluxing into the medium around brain slices as they undergo ischaemia–reperfusion injury are supportive[74]. In these images, the regions showing maximal release include the pyramidal cell stratum, which contains only pyramidal neuron somata (FIG. 3).

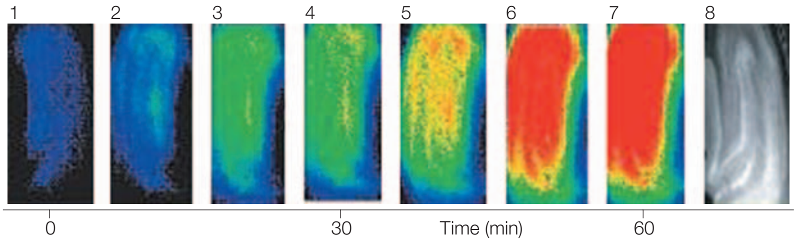

Figure 3 | **Zinc release into the extracellular fluid of a hippocampal slice induced by nitric oxide.** The 60-min experiment begins in (1). A zinc-sensing fluorescent probe is present in the extracellular fluid, and shows essentially no extracellular zinc in frame 1 (baseline), then shows increasing amounts being released in frames 2–4, as the oxygen–glucose deprivation proceeds. Immediately after reperfusion, zinc efflux is enhanced (oxygen–glucose replacement; frame 4), and reaches a maximal after 30 min of reperfusion (frame 7, 60 min). The entire zinc release in this oxygen–glucose deprivation paradigm can be blocked by inhibition of nitric oxide synthase by L-NAME (N(omega)-nitro L-arginine methylester). The hippocampal slice is shown in (8) in brightfield. Modified, with permission, from REF. 74 © (2004) International Brain Research Organization.

So far, the NO–MT–Zn^{2+} signalling cascade has been observed only in pathological situations, such as status epilepticus, trauma or ischaemia–reperfusion[75,76,77]. However, it is possible that this pathway also operates at a reduced level in the healthy brain[18]. The coupling of NMDA receptors to neuronal nitric oxide synthase (nNOS)[78,79] could mediate this putative pathway.

Transporters

Two groups of proteins that are involved in zinc transport are known; the divalent cation transporter (DCT) family[80] and the zinc transporter (ZnT) family[81]. However, the ZnT proteins might only modulate transport indirectly[82], whereas the DCT family have been shown to be direct transporters[72,80]. The elucidation of the ways in which these proteins cooperate to regulate zinc metabolism and signalling will, no doubt, be a fascinating new chapter in the neurobiology of zinc.

Physiological functions of zinc signals. Research on the neuropharmacology of zinc signals is hindered by the fact that zinc is an ion, not a molecule. Therefore, there are no synthetic or metabolic enzymes to inhibit or stimulate, nor any receptor agonists or antagonists that can be deployed. Furthermore, in any biologically-relevant medium near normal pH, exogenous zinc will precipitate as zinc hydroxide complexes, or bind to myriad ligands in the medium and tissue, which means that the actual free zinc signal produced is often less than 0.1% of the total zinc added[83]. However, proper control of the free zinc concentration (pZn) is possible using pZn buffers[84–86] and measuring methods[87].

Excitatory amino acid receptors. The NMDA-type glutamate ionophore was found to be inhibited by zinc in 1987 (REF. 88). The zinc-sensitivity of this molecule is now understood to be mediated by two separate mechanisms: a voltage-independent site on the NR2A subunit that has an IC_{50} in the single-digit nanomolar range[86,89], and a less sensitive, voltage-dependent site on the NR2B[90] subunit, where ionic current is depressed by low-micromolar concentrations of zinc.

The high-affinity site on NR2A is especially interesting, because the apparent extracellular pZn of healthy brain tissue is between 8 and 9 (that is, the concentration of free Zn^{2+} is between 1 nM and 10 nM). This implies that the zinc site on NR2A is normally partially occupied by zinc, and that the NMDA channel current is correspondingly depressed. This, in turn, implies that merely chelating the extracellular zinc to above pZn = 10 (free zinc concentration below 0.1 nM) should increase the excitability of the exposed brain tissue. Several investigators have observed that the introduction of a sufficiently high-affinity zinc chelator leads to increased amplitude of NMDA-mediated postsynaptic responses[84,91,92], increased excitability and/or lowered threshold for seizure induction[93–96].

The inhibitory effect of zinc on the NR2A subunit is synergistic with the inhibitory effect of protons, with zinc shifting the pH sensitivity of NR2A towards stronger inhibition at a given pH[89]. So, the maximum depression of NMDA currents occurs when extracellular pH and pZn^{2+} are simultaneously falling. However, the on-rate and, in particular, the off-rate of Zn^{2+}–NR2A binding is slow[89], so a relative change will be seen in this tonic downregulation of the NR2A subunit when the zinc concentration changes. This probably explains why brief 'puffs' of zinc fail to alter NMDA-gated currents[97], whereas zinc chelation relieves zinc inhibition[84,91–96].

Zinc also causes a paradoxical delayed increase in the sensitivity of the NMDA receptors to agonists. This delayed effect (over hours) is mediated by increased tyrosine phosphorylation of the NR2A and NR2B subunits, which decreases their sensitivity to zinc-mediated tonic inhibition[98,99]. This negative feedback results in a net potentiation of synaptic currents, which is mediated by the NMDA receptor. A similar long-term potentiation (LTP) of the glutamate synapse through exposure to Zn^{2+} has been shown at the mossy fibre–CA3 synapse, where the zinc seems to function intracellularly in the CA3 pyramidal neurons[18].

Recent detailed analysis showed that ~45% of all dendritic spines in the stratum radiatum of the hippocampus receive zinc-containing glutamatergic synaptic input, whereas ~55% receive zinc-free glutamatergic input[27]. Intriguingly, the zinc-containing inputs preferentially innervate postsynaptic sites with NMDA-type receptors, as opposed to AMPA or kainate receptors[27]. This implies that the zinc-mediated downregulation of NMDA receptors is based on local release of zinc from the immediately adjacent presynaptic terminal.

Inhibitory amino acid receptors. The second receptor to be studied intensively for zinc sensitivity was the $GABA_A$ (GABA type A) receptor[100]. Two decades of elegant work by Smart and colleagues[17,101] and others[102–104] have culminated in almost complete explication of the molecular mechanisms by which zinc modulates $GABA_A$ receptors[105]. The $\alpha1\beta3$ splice variant is most sensitive to zinc: other α and β variants have lower sensitivity, and $GABA_A$ receptors that contain γ subunits have greatly reduced sensitivity, owing to the

Table 1 | **Zinc-sensitive targets in the central nervous system**

Specific protein	Main effect of zinc	References
Glutamate receptors		
AMPA	Up- and downregulates	254
NMDA	Tonic downregulation Phasic effect disputed	99,255
Metabotropic	Downregulates	256
Other receptors		
$GABA_A$	Mostly downregulates	17,257
$GABA_B$	Mimics, downregulates	258
Glycine	Upregulates	125
Sigma 2	Mimics	259,260
Acetylcholine	Up- and downregulates	124
Adenosine	Up- and downregulates	121
Serotonin	Downregulates	261
Dopamine	Up- and downregulates	117,118
Catecholamine	Up- and downregulates	120
Melanocortin	Upregulates	262
Zinc receptor	Mobilizes intracellular Ca^{2+}	134
Proton receptor	Zinc–proton synergy	89
Opioid	Downregulates	263
Channels		
Ca^{2+}	Blocks, inhibits	264
K^+	Up- and downregulates	265
Na^+	Mixed	102,266
Cl^-	Facilitates	267
Ca^{2+}-amyloid	Blocks	268
Transporters		
Glutamate	Decreases uptake	135,269
Dopamine	Decreases uptake	136,270

AMPA, α-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid; $GABA_A$/$GABA_B$, γ-aminobutyric acid type A/B; NMDA, *N*-methyl-D-aspartate.

interposition of the γ subunit, which disrupts the α–β interface site[105].

Several exemplary experiments have used the blockade (chelation) protocol to reveal the effects of endogenous zinc signals on GABA receptors[106–108]. Because there are neurons in the spinal cord that release GABA along with zinc[109], the modulation of GABA receptors by zinc is probably a vital factor in normal brain function .

Changes in the zinc modulation of GABA receptors have been implicated in the aetiology of epilepsy. Mody, Coulter and others[110–112] have suggested that the seizure-induced sprouting of zinc-releasing axons into ectopic locations could result in ectopic release of zinc, thereby reducing $GABA_A$ receptor-mediated inhibition and enhancing seizure susceptibility[111]. The release of $GABA_A$ receptor-inhibiting quantities of zinc in the brains of rats with a history of seizures has not yet been found[113], but this hypothesis remains attractive. In addition to the sprouting of zinc-releasing axons, further changes in the zinc modulation of GABA receptors might contribute to progressive epileptogenesis[114]. GABA-receptor modulation by zinc also changes dramatically during early brain development[115], and in the adult circadian pacemaker region as a function of the circadian cycle[116].

Other receptors, channels and transporters. Zinc has been proposed to affect aminergic[117–120], purinergic[121,122] and cholinergic[123,124] receptors, but the physiological importance of such putative effects remains uncertain. It has also recently been shown that the glycine[125] and proton[126,127] receptors are sensitive to zinc. Given the strong evidence that glycine and zinc co-localize in presynaptic terminals in the brain stem and spinal cord[128], the fact that zinc inhibits glycine receptors at high concentrations (>10 μM) and facilitates them at slightly lower concentrations (<10 μM) might have physiological significance[129–131]. The co-activation of acid-sensing proton receptors by zinc could also be important, especially in excitotoxic brain injury scenarios, in which both extracellular pZn and pH are likely to fall[127,132,133].

The recently-described zinc-sensing receptor, which is a membrane-spanning protein that is sensitive to zinc under physiological conditions[134], also merits further attention. This receptor, which has been described on epithelial cells, initiates calcium mobilization through calcium/calmodulin dependent protein kinase activation of cell growth and proliferation, thereby giving the Zn^{2+} signal potent control over the fate of skin tissue[134]. Similar zinc sensors might be present in other tissues, including the brain.

Several types of voltage-gated channels and transporters have also been shown to be sensitive to exogenous zinc (TABLE 1). Intriguing examples include the inhibition of glutamate-uptake transporters by zinc[135] (another mechanism by which zinc could modulate glutamatergic synaptic transmission), and the effects of zinc on the cocaine-sensitive site of dopamine-reuptake transporters (a potential therapeutic target for the treatment of cocaine abuse)[136].

Zinc and brain function

Brain excitability. Zinc inhibits both excitatory and inhibitory receptors, so, in principle, it could make the forebrain neurons more excitable, less excitable or have no net effect. However, the administration of zinc chelators has generally produced outright paroxysmal/epileptiform brain activity, lowered the threshold for seizure induction, or increased the excitatory postsynaptic potentials (EPSPs) or excitatory postsynaptic currents (EPSCs) at NMDA receptor synapses, which indicates that the dominant effect of Zn^{2+} in the normal brain is to reduce excitability, thereby functioning as an endogenous anticonvulsant. Unfortunately, the converse treatment, which involves intracranial administration of zinc salts, is directly cytolethal and proconvulsive[137] (but see REF. 138).

Synaptic plasticity. The conspicuous concentration of glutamate- and zinc-releasing terminals in the neocortex and limbic structures (the septum and amygdala) indicates that glutamate- and zinc-releasing synapses might have a special role in the synaptic plasticity that underlies learning and memory[139,140]. It has been suggested that both developmental and experiential plasticity are zinc-dependent.

The plasticity of the young mammalian brain is frequently accompanied by changes in innervation by zinc-containing neurons. For example, the differentiation of striosomes in the caudate–putamen is first signalled by the appearance of zinc-containing boutons in each striosome[141], followed by innervation that separates the striosomes from the matrix. A similar example can be observed in the lateral geniculate nucleus, where zinc-containing boutons appear briefly when the nucleus undergoes reorganization after denervation[142]. Furthermore, in visual and somatosensory cortical areas, zinc-containing innervation is an early marker for the various columns and barrels that delineate sensory fields[19,143], and changes in the early sensory experience are reflected in changes in the pattern of zinc-containing innervation[19].

The idea that glutamate- and zinc-releasing synapses might have a zinc-dependent mode of experiential plasticity has been tested repeatedly, with mixed results. The role of zinc in LTP at the mossy fibre–pyramidal synapse, where the giant zinc-filled mossy boutons contact the CA3 pyramidal neurons, has been studied by five groups. Three groups found no change in LTP as a result of zinc chelation[37,92,139], whereas the fourth found that blocking zinc signalling blocked LTP[144] and the fifth found that LTP could be blocked or induced by zinc chelation or delivery, respectively[17]. The differences in preparation and methods that account for these discrepancies remain to be established.

Acute toxicity of free zinc

Although zinc lacks redox activity and has traditionally been regarded as relatively non-toxic[145], there is increasing evidence that free ionic zinc is a potent killer of neurons and glia. Yokoyama and associates[146] showed that 15 min exposure to 300–600 μM zinc results in extensive neuronal death in cortical cell culture. Combined with the discovery that neurons store up to 300 μM of free zinc in their terminals[147] and release zinc when they are depolarized[29,38,39], these findings indicated that zinc has an active role in neuronal injury.

This possibility was strengthened by the observation that membrane depolarization — which invariably accompanies acute brain injury[148,149] — greatly increases the potency of zinc as a neurotoxin. For instance, in cortical cell culture, depolarization with high concentration (25 mM) potassium media allows just a 5-min exposure to 100 μM zinc to kill most neurons[150]. This mechanism of increased toxicity probably involves zinc influx, and subsequent calcium influx, through L-type calcium channels. NMDA and calcium-permeable AMPA/kainate channels might also provide routes for zinc entry[59,70,151].

Recent work using zinc-buffered cell growth media has shown that eukaryotic cells die if grown in media that contains free zinc in excess of ~100 nM (pZn = 7)[83]. Preliminary estimates indicate that the physiological $[Zn^{2+}]_i$ in eukaryotic cells is in the low picomolar range (pZn ~12.5)[43]. When the $[Zn^{2+}]_i$ falls to the levels that are induced by strong chelators (pZn>15), apoptosis can be triggered[152]. When the $[Zn^{2+}]_i$ rises to nanomolar concentrations (pZn< 9), toxicity ensues[56,153].

***Zinc toxicity* in vivo.** The idea that zinc toxicity could contribute to neuronal injury *in vivo* was first suggested in 1988, on the basis of findings in rats that had undergone prolonged seizures or transient cerebral ischaemia[30,154,155]. Staining of adjacent brain sections from these animals with TSQ and acid fuchsin revealed a striking correlation between zinc accumulation in cell bodies and cell death. It was shown later that both neuronal death and zinc accumulation in transient cerebral ischaemia were reduced or prevented by the zinc chelator calcium edetate (CaEDTA), but not by the non-zinc chelator zinc edetate (ZnEDTA)[156]. Subsequently, the principle of endogenous zinc toxicity as a contributing mechanism has been investigated and shown to be valid in other injury models, including blunt head trauma[33], focal ischaemia[157], oxygen–glucose deprivation *in vitro*[158] and glucose deprivation *in vivo*[159].

Zinc translocation. Vesicular zinc was initially thought to be the only releasable pool of zinc[154], and it was assumed that the zinc that appears in injured neurons was probably of presynaptic origin[154]. The later discovery that zinc could enter neurons through various voltage- and glutamate-gated channels supported this hypothesis[7,71], as did the discovery that the membrane-impermeant zinc chelator CaEDTA substantially reduced zinc accumulation in degenerating neurons[33,156,157]. Because CaEDTA remains in the extracellular space, this finding was taken to be consistent with the chelation of released zinc in the extracellular space.

If presynaptically-released zinc were the only source of toxic zinc that contributed to the degeneration of postsynaptic neurons, animals with no presynaptic zinc should not be susceptible to such zinc toxicity in excitotoxic brain injury situations. Mice that lack the zinc transporter ZnT3 show no histochemically reactive zinc in their presynaptic vesicles, and this is associated with a substantial reduction in the amount of neuronal zinc staining and neuronal death in the CA3 region of the hippocampal formation, where synaptic release of zinc from the mossy boutons is extensive[160]. However, other brain regions in which the synaptic zinc input is scanty did not show diminished zinc staining in these mutants, which indicates that the zinc in injured neurons comes from both presynaptic and other sources.

Other toxic mechanisms. The 'zinc-translocation' hypothesis is now recognized as incomplete[69] for several reasons. First, zinc accumulation in degenerating neurons is always observed, to some extent, in areas that are only lightly innervated by glutamate- and zinc-releasing fibres. For example, thalamic neurons are surrounded by terminals that lack vesicular zinc[13,154], but still show zinc accumulation following ischaemia or seizures[156]. Second, even in *Znt3*-null mice, extensive zinc accumulation has been observed in degenerating CA1 and thalamic neurons[160]. Last, the recent discovery that extracellular CaEDTA can remove zinc from inside cells and even presynaptic vesicles[161] indicated that zinc blockade by CaEDTA could no longer be accepted as evidence that the zinc had travelled through the extracellular fluids.

Zinc accumulation in degenerating neurons of *Znt3*-null mice indicates that there are other dynamic zinc sources besides that found in synaptic vesicles. Zinc can be mobilized from MT3, and possibly also from mitochondria, and this intracellular zinc release could lead to a somatic release of zinc into the extracellular fluid with subsequent zinc translocation into neighbouring cells. The direct role of nitric oxide in releasing this MT3 pool of zinc during excitotoxicity was recently shown by Wei[74] (FIG. 3) and others[76,77] (for a review, see REF. 75).

Zinc-initiated cell death pathways

Initially, the toxic effect of zinc was puzzling, because zinc had been considered to be a relatively innocuous metal and was known to inhibit apoptosis in diverse cell systems[162].

Although zinc is not itself an oxidant, several lines of evidence indicate that zinc toxicity is mediated largely by oxidative stress. First, zinc-induced cell death is accompanied by increased levels of superoxides and lipoperoxides, which are markers for oxidative injury[163–165]. Second, zinc-induced cell death is attenuated by various antioxidative measures[166,167]. Last, free-radical-generating enzymes, such as NADPH oxidase, are induced and activated after zinc exposure, and their inhibitors attenuate zinc toxicity[168].

Zinc-induced apoptosis. Neurons that are briefly exposed to high concentrations of zinc show signs of necrosis, such as cell body swelling and destruction of intracellular organelles[163]. However, under conditions of less fulminant zinc toxicity, signs of apoptosis, such as DNA fragmentation and caspase activation, are observed[166,169].

The mechanisms for zinc-triggered apoptosis are now being identified. In zinc-exposed neurons, both the neurotrophin receptor $p75^{NTR}$ and $p75^{NTR}$-associated death executor (NADE) are induced[170], which is a combination that can trigger caspase activation and apoptosis[171]. In addition, zinc can trigger the release of pro-apoptotic proteins, such as cytochrome *c* and apoptosis-inducing factor (AIF), from mitochondria[172]. It is not known to what extent apoptosis contributes to zinc-related acute brain injury, but in rat models of ischaemia or seizures, in which zinc is likely to function as a neurotoxin, $p75^{NTR}$ and NADE are co-induced in neurons that undergo cell death[170,173].

Nitric oxide and zinc toxicity. Nitric oxide has a pivotal role in zinc toxicity. It releases seven zinc ions from each MT molecule[77], and the brain-specific MT3 isoform has a considerably lower threshold for zinc release by nitric oxide than the other isoforms[174]. Inhibition of nitric oxide synthase (NOS) markedly reduces the release of zinc from brain slices[74] and reduces the appearance of zinc staining after traumatic or epileptic brain injury (C.J.F. and R. Masalha, unpublished observations) or hypoglycaemic brain injury[158], so it is clear that nitric oxide-mediated release of zinc from MT has a crucial excitotoxic role. Nitric oxide also rapidly releases zinc from presynaptic terminals[175], thereby contributing to cell death through the zinc-translocation mechanism. Furthermore, elevated intracellular zinc induces and activates nNOS in cultured cortical neurons[176], so zinc and nitric oxide can both trigger a destructive cycle.

Poly-ADP-ribose polymerase. The final pathway to zinc-induced cell necrosis seems to occur through poly-ADP-ribose polymerase (PARP) activation, which has been shown in other cases of predominantly necrotic cell death[177]. DNA damage induced by oxidative and nitrative stresses activates PARP, which transfers the ADP-ribose moiety from nicotinamide adenine dinucleotide (NAD^+) to various target proteins. As up to several hundred moieties are transferred to one protein molecule, continued activation of PARP results in a drastic depletion of NAD^+ and ATP[178]. Consistent with the idea that PARP activation is limited to necrosis[177], induction of apoptosis by chronic exposure to low concentrations of zinc[169] is not attenuated by the deletion of PARP1 (REF. 179).

Zinc and neurodegenerative disease

Alzheimer's disease. One of the pathological hallmarks of Alzheimer's disease is the marked accumulation of amyloid-β (Aβ) protein, in the form of senile plaques and cerebrovascular amyloid deposits[180–182]. There is considerable evidence that free zinc in the extracellular fluid induces amyloid deposition[183,184] (FIGS 4 and 5), and early-phase clinical trials indicate that zinc chelation inhibits Aβ-plaque deposition[185,186].

The Aβ peptide is produced from the proteolytic cleavage of amyloid precursor protein (APP)[182]. A specific and saturable binding site for zinc (K_D = 750 nM) has been reported in the cysteine-rich region on the ectodomain of APP[187,188]. This site has homology to all known members of the APP superfamily and the amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2)[187], which indicates that zinc interaction might have an important, evolutionarily conserved role in APP function and metabolism. Many observations indicate a role for zinc in sustaining the adhesiveness of APP during cell–cell and cell–matrix interactions[189,190].

Aβ40 specifically and saturably binds zinc, manifesting higher-affinity binding (K_D = 107 nM) with a 1:1 (zinc:Aβ) stoichiometry and lower-affinity binding (K_D = 5.2 μM) with a 2:1 stoichiometry[183,184].

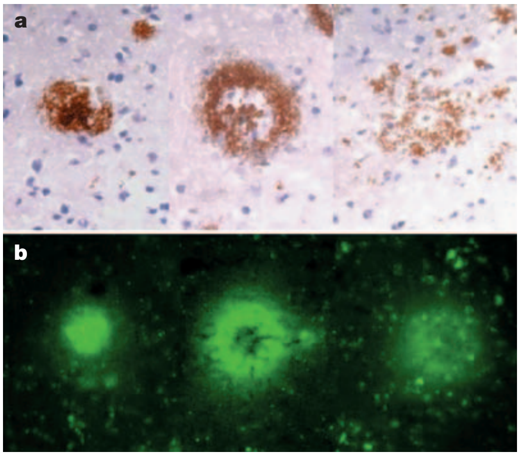

Figure 4 | **Zinc in the amyloid-β plaques of Alzheimer's disease.** Three cortical senile plaques from post-mortem human brains are immunostained for amyloid-β (**a**), and three similar plaques are stained for zinc using *N*-(6-methoxy-8-quinolyl)-p-toluenesulphonamide (TSQ) fluorescence (**b**). Reproduced, with permission, from REF. 204 © (2003) Elsevier Science.

Because the pZn of the extracellular brain milieu is apparently in the 8–9 range ($[Zn^{2+}]$ ~1–10 nM), it would be expected that Aβ40 would bind little zinc under normal conditions. However, events that lead to a sustained decrease in pZn owing to a sustained release of zinc from cells, such as a transient hypoperfusion, head trauma or even local paroxysmal neuronal firing[75], could lead to the saturation of the higher- and (potentially) the lower-affinity zinc sites. Zinc release in excess of 100 nM (pZn = 7) has been observed in such circumstances[43].

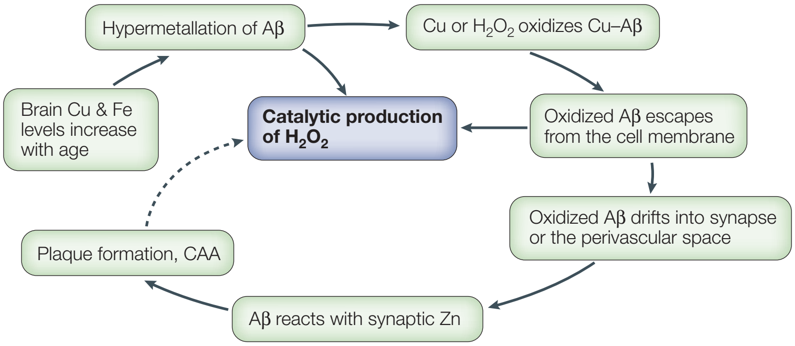

Figure 5 | **Proposed model for pathogenic copper and zinc interaction with amyloid-β in Alzheimer's disease.** Copper and iron levels increase in specific subcellular compartments of the brain with age. Amyloid-β (Aβ) becomes overwhelmed in its attempt to contain or transport copper, and becomes oxidized by copper or hydrogen peroxide (H_2O_2). This leads to protease-resistant dityrosine species, as well as oxidation of methionine 35 on Aβ, which allows Aβ to escape its constitutive membrane compartment. This drives up the levels of soluble Aβ (monomeric and oligomeric) in the brain. When bound to copper, these forms are toxic owing to their redox activity and the catalytic generation of H_2O_2. These soluble Aβ species drift into the interstitial spaces of the brain, where they are driven out of solution by the exceptionally high concentrations of zinc influx at the glutamatergic synapses and the perivascular spaces, resulting in plaque formation and cerebral amyloid angiopathy (CAA). Although the zinc partially quenches Aβ-mediated redox activity, the amyloid deposits are still sites of considerable H_2O_2 formation and oxidation.

The zinc-binding site in Aβ40 has been mapped to a stretch of contiguous residues between amino-acid residue positions 6 and 28, and the histidine residue at position 13 seems to have a crucial role in zinc-mediated aggregation[191]. Occupation of the zinc binding site[192] inhibits α-secretase-type cleavage, and might influence the generation of Aβ from APP, as well as increasing the biological half-life of Aβ by protecting the peptide from proteolytic attack[183]. Zinc rapidly precipitates synthetic human Aβ40 (REF. 184), and chelation treatment completely reverses this precipitation[193].

Although zinc-induced Aβ precipitation at pH 7.4 is highly specific to zinc, copper and iron can also induce partial aggregation, which increases substantially under mildly acidic conditions (pH 6.6)[194]. Zinc, copper and iron are all markedly enriched in amyloid plaques[195] (TABLE 2), but only copper and zinc co-purify with the Aβ extracted from post-mortem human brains[196] and have been shown to coordinate with Aβ in plaques[197].

There is considerable indirect evidence that APP and Aβ might function as copper chaperones or effluxers[183,184,194,198]. In addition, knockout mice that lack either APP or APLP2 show specific elevations in brain and liver copper levels[199], whereas overexpression of APP or of APP's 100-amino-acid carboxy-terminal (APP-C100) fragment results in decreased copper levels[200,201]. Studies in yeast, as well as primary neuronal cultures from APP-knockout mice and APP-transgenic mice[202], confirm that APP and Aβ expression mediates the export of a significant fraction of neuronal copper.

In the mouse brain, copper and iron levels increase with age[203]. One idea to explain this is that Aβ (REF. 198) becomes hypermetallated with age, and is abnormally oxidized during the physiological processing of copper[204]. Abnormal binding of copper to Aβ could yield two adverse outcomes: toxicity, mediated by redox activity, and oxidative modification of Aβ. Aβ–Cu^{2+} complexes are strongly reductive, and generate hydrogen peroxide catalytically from biological reducing agents, including cholesterol[196,205,206]. The redox activity is stronger for human Aβ42 than for human Aβ40 or the rat Aβ peptide, which correlates with the toxicity of the peptide in cell culture[207]. Copper-mediated oxidation of Aβ causes damage to histidine and tyrosine side-chains[208], dityrosine crosslinking[209] and sulphoxidation of the sole methionine residue that is located at position 35 (REF. 210). This methionine residue is essential for keeping metallated Aβ in its normal (redox-silent) location within lipid membranes[211,210]. Therefore, oxidation of Aβ by copper might be the first step in the liberation of soluble Aβ species that can later be precipitated by zinc (FIG. 4). This might explain why almost all the Aβ deposits found in the brains of individuals with Alzheimer's disease are oxidized[213]. The generation of hydrogen peroxide by soluble but oxidized forms[214] of Aβ might explain the association of brain Aβ accumulation with the severe peroxidative damage that is characteristic in the brains of individuals with Alzheimer's disease[215] and of APP transgenic mice[216]. Zinc and copper chelators reverse Zn/Cu-induced aggregation of synthetic Aβ *in vitro*[217], inhibit

Table 2 | **Metal levels in patients with Alzheimer's disease and healthy individuals**

Location	Zinc μg g^{-1} (μM)*	Copper μg g^{-1} (μM)*	Iron μg g^{-1} (μM)*
Plaque rim	67 (1024)‡	23 (357)‡	52 (938)‡
Plaque core	87 (1327)‡	30 (474)	53 (951)‡
Total senile plaque	69 (1055)‡	25 (393)‡	53 (940)‡
Alzheimer's neuropil	51 (786)§	19 (304)	39 (695)
Control neuropil	23 (346)	4 (69)	19 (338)

*Numbers in brackets represent molar concentrations, which were converted with the assumption of a sample density equivalent to 1 g cm^{-3}; ‡$p<0.05$ (plaque values compared with neuropils from patients with Alzheimer's disease); §$p<0.05$ (neuropils from patients with Alzheimer's disease compared with neuropils from control individuals). Adapted from REF. 195.

Aβ-mediated hydrogen peroxide formation[196,206] and solubilize Aβ from amyloid deposits in post-mortem brain tissue from patients with Alzheimer's disease[215].

Studies of the impact of the genetic ablation of ZnT3 in the Tg2576 mouse model of Alzheimer's disease have provided evidence that synaptically released zinc underlies amyloid pathology. We found that the complete absence of any staining for synaptic vesicle zinc in the knockout mouse was accompanied by a profound reduction in the cerebral plaque load[216]. Both synaptic zinc levels and plaque burden increased to a greater degree with age in female compared with male mice, which indicates that sex hormones influence synaptic zinc levels[216]. Preliminary evidence indicates that oestrogen might reduce the level of synaptic vesicle zinc, perhaps by modulating the expression level of the adaptor protein 3 (AP3) complex, which is required for the correct insertion of ZnT3 into vesicular membranes[216]. Cerebral amyloid angiopathy is also decreased in ZnT3-knockout Tg2576 mice compared with Tg2576 controls, which indicates that there might be a ZnT3-dependent communication of plasma and neuronal zinc through the cerebrovascular walls[217].

Besides the direct effect of the pZn on amyloid aggregation, it is also possible that zinc contributes to the pathology of Alzheimer's disease through interaction with other zinc-dependent or zinc-containing proteins. Considering that ~3% of all proteins contain zinc-binding motifs, this is a likely prospect. Potential candidates that might have an indirect, zinc-related role in Alzheimer's disease include α_2-macroglobulin, nerve growth factor-β (NGFβ), S100 calcium-binding protein β (S100β), metallothionein and zinc-dependent proteases. Several reports have indicated that in neocortical tissue that is affected by Alzheimer's disease, zinc levels rise in excess of the molar increase of Aβ (for a review, see REF. 14). Tissue fractionation studies to elaborate this elevation have not yet been reported, but it is probable that several proteins will have increased zinc stoichiometry in advanced Alzheimer's disease.

Amyotrophic lateral sclerosis. Two abnormalities of zinc-metalloproteins are implicated in the pathophysiology of amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease). First is the well-established fact that the familial form of ALS is caused by mutations in the metalloenzyme Cu/Zn-superoxide dismutase (SOD)[218,219]. Mutations in SOD are associated with ALS-like spinal motor defects in mice, and different mutants have different amounts of wild-type enzymatic activity, which range from 0% (arginine substituted for histidine at amino acid position 46 (His46Arg) and Gly85Arg) to 100% (Gly37Arg). SOD1-knockout mice do not develop the ALS phenotype[220], and the age of onset and duration of disease in ALS-transgenic mice is unaffected by levels of wild-type SOD1 activity[221], which indicates that the toxicity of mutant SOD1 is the result of a gain of function.

Several gain-of-function redox reactions have been proposed for mutant SOD1, and, currently, at least two seem plausible. Increased peroxidase activity has been reported *in vitro*[221,222] in the His48Gln, Ala4Val, and Gly93Ala variants, although not consistently[222]. Increased peroxidase activity *in vivo* has been reported in the Ala4Val and Gly93Ala[223] species. Copper-replete, zinc-deficient SOD1 has been reported to confer toxicity by producing peroxynitrite according to these reactions, and loss of zinc from mutant SOD1 has been proposed to be a primary pathogenic event[224].

The second zinc metalloprotein that is aberrant in patients with ALS is metallothionein, immunoreactivity to which is elevated in the brain and liver[218,219]. The same pattern occurs in a transgenic-mouse model of ALS: SOD1-Gly93Ala-transgenic mice show increased MT1, MT2 and MT3 expression in astrocytes and increased MT3 in neurons[225]. Metallothionein elevation is probably compensatory (for example, in response to oxidative stress) and protective. In the Gly93Ala mutant SOD1 transgenic model of ALS, deficiency of MT1, MT2 or MT3 exacerbates the ALS phenotype[226,227].

Zinc as a therapeutic target

Whether the aim is to treat an acute, toxic excess of free zinc, as occurs in excitotoxic brain injury, or to treat a possible chronic elevation of free zinc, as might occur in Alzheimer's disease, the pZn of the brain must be maintained within physiological limits. As mentioned above, the pZn of the mammalian brain seems to be in the range of 10–20 nM[43], and deviations substantially above or below this range are proconvulsive and cytolethal, respectively (FIG. 6).

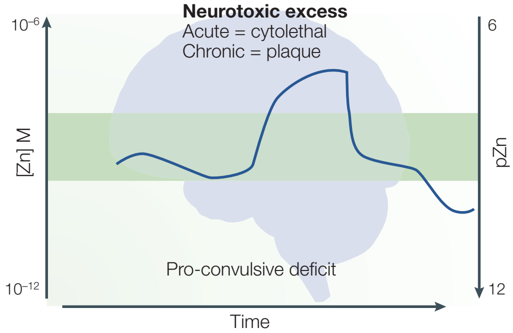

Figure 6 | **Extracellular zinc buffering.** The concentration of free zinc in the brain is normally low, in the 1 to 10 nM range (pZn = 8–9). During excitotoxic insults (such as stroke, cardiac arrest, head trauma or seizures), pZn falls, and neurons are at risk of zinc-induced toxicity. Alternatively, if the zinc concentration falls too low (pZn ≫ 10), there is increased excitability in the cortical circuitry, and, if deficiency is maintained for too long, deficiency-induced apoptosis. Buffering can control pZn, both *in vivo* and *in vitro*, which prevents these damaging processes.

Upstream regulation of free zinc. One novel approach to controlling pZn would be to slow or reduce the release of free zinc. As nitric oxide seems to trigger much of the zinc release that occurs in injury scenarios (see above), inhibiting whichever NOS is responsible for the zinc-releasing nitric oxide is a plausible approach to reducing zinc-induced brain injury. Inhibition of nNOS has shown promise in reducing both the amount of zinc released and the number of zinc-staining neurons (and, therefore, potentially the number of injured neurons) after excitotoxic injury[74,75,159].

Buffering free zinc. There are three options for zinc-based drug development. First, zinc buffers with equilibrium constants in the 10^{-8} to 10^{-9} range would maintain pZn in the optimal range (9>pZn>8), thereby preventing excess zinc damage while avoiding a harmful degree of zinc deficiency. Second, for acute brain injuries (for example, stroke, trauma, ischaemia and hypoperfusion), short-lived chelation with compounds that have higher binding affinity might allow some control of zinc toxicity with minimal deleterious effects of lowered zinc. Last, 'pro-buffers' or 'tethered buffers' could be targeted towards specific cytological compartments, acting on zinc only when or where such zinc buffering is therapeutic.

The strategy of using a relatively weak chelator has already produced promising results in both animal and human studies of Alzheimer's disease. The quinoline compound clioquinol — a lipophilic chelator that crosses the blood–brain barrier — binds zinc in the mid-nanomolar range. Oral clioquinol has been shown to dramatically reduce the amount of amyloid plaques in transgenic mice and to slow the rate of cognitive decline in patients with Alzheimer's disease[185,186,202].

Another promising use of the low-affinity approach has been reported for the excitotoxic, acute zinc-toxicity syndrome in which the zinc ionophore, pyrithione, can rescue cultured cells from zinc toxicity if administered at the right time[228]. Pyrithione presumably transports free zinc down its concentration gradient across the membrane, thereby rescuing cells from zinc toxicity when intracellular pZn is lower than extracellular pZn. Unfortunately (but not unexpectedly), pyrithione exacerbates zinc toxicity if applied when the extracellular pZn is lower than the intracellular pZn[43].

The idea of a 'pro-drug' chelator is also under active investigation as a treatment for Alzheimer's disease. A classical strong chelator (BAPTA) is rendered lipophilic and inactive by the addition of alkyl chains. Once through the blood–brain barrier and embedded in a cell wall (lipid membrane), the pro-drug (DP-109) can be transformed into active BAPTA by membrane lipases. It is, therefore, expected that DP-109 will chelate metals predominantly in the vicinity of cell membranes. In Tg2576 mice, DP-109 significantly reduced Aβ-plaque load by ~60–80% without noticeable side effects[229] (FIG. 7). The related compound DPb99 has also proved efficacious in small samples of human patients as a neuroprotectant against the zinc-mediated injury that is caused by stroke and during bypass surgery[230].

Downstream control of zinc-triggered signals. Therapies that target later events are also promising. As discussed above, diverse serial and parallel events contribute to zinc-induced cell death. First, as zinc toxicity is largely mediated by oxidative and nitrosative stress[8,163,165,176,], antioxidants and NOS inhibitors might be useful. Second, the targeted inhibition of PARP[179,198] might be effective in reducing zinc toxicity. Third, anti-apoptosis measures, such as caspase inhibition, might be a possibility. Although these mechanisms have been shown to contribute to zinc toxicity in cell culture, they are considered more or less general mechanisms of cell death in acute brain injury. At present, it is not known whether any particular neuroprotectant is more effective against zinc toxicity than other injury mechanisms. As a result, more studies might be needed to identify drug targets that are more specific to zinc toxicity. NADPH oxidase might be such a target, because it is induced and activated during zinc toxicity but much less so during calcium excitotoxicity[231].

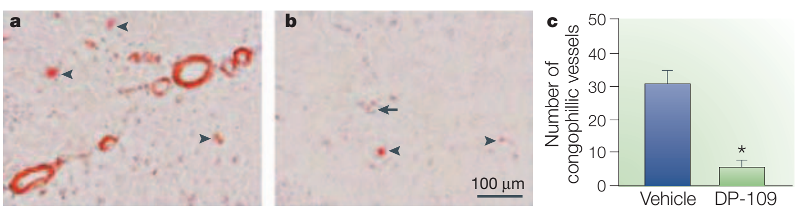

Figure 7 | **Effect of zinc and copper chelation on amyloid neuropathology in a transgenic mouse model of Alzheimer's disease.** Tg2576 mice were given either the control vehicle (**a**) or the metal chelator DP-109 (**b**, 5 mg kg^{-1}) daily for 3 months, after which their brains were removed and assayed for congophilic amyloid deposition around blood vessels (arrows) and parenchyma (arrowheads). Sections show Congo red-stained cortex. The number of congophilic vessels per brain section is shown in **c**. Mean, n = 13; standard error of the mean, n = 15); *significant difference (p<0.01). Reproduced, with permission, from REF. 229 © (2004) Elsevier Science.

Pyruvate protects against zinc-induced cell death in cortical and oligodendrocyte progenitor cell cultures[232]. Pyruvate protection is quite specific to zinc toxicity, because pyruvate does not attenuate calcium-overload excitotoxicity in the same cortical cell culture[233]. Consistently, in a rat model of transient global ischaemia in which the role of zinc is established[155,156], pyruvate almost completely blocks zinc accumulation as well as neuronal death throughout the brain. A direct antioxidative effect and/or normalization of NAD^+ levels might contribute to cytoprotection by pyruvate[234,235].

Another possible neuroprotectant with specificity against zinc-mediated injury is tissue plasminogen activator (tPA), which is currently used for thrombolysis in human patients[236]. Although most of tPA's biological effect is mediated by its protease activity[237], blockade of zinc toxicity by tPA takes place even in the presence of excess protease inhibitors[238]. Although the protective mechanism is still unclear, tPA does not seem to function by reducing extracellular zinc or zinc influx into cells[239]. A preliminary result indicates that certain membrane receptors with tyrosine kinase activity might mediate this effect, as the epidermal growth factor receptor tyrosine kinase inhibitor C56 can reverse the protection (J. Y. Koh, unpublished observations). If the effective moiety and its cognate membrane receptors can be identified, development of tPA-derived peptides that prevent zinc toxicity might be possible.

Conclusions and future directions

Like calcium, zinc is proving to be an essential and ubiquitous ionic signal in a myriad of cells and tissues. Because fluorescent calcium probes frequently respond to zinc as well, separating calcium signals from zinc signals will be mandatory in future research. Therapies based on manipulating zinc signals by preventing release, blocking channels, altering transport and buffering the pZn of target tissues are all likely to have increasingly important roles in twenty-first century medicine.

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Competing interests statement

The authors declare competing financial interests: see Web verson for details

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