Copper-dependent regulation of NMDA receptors by cellular prion protein: implications for neurodegenerative disorders

Peter K. Stys[1], Haitao You[2] and Gerald W. Zamponi[2]

[1] *Departments of Clinical Neurosciences and* [2] *Physiology & Pharmacology, Hotchkiss Brain Institute, University of Calgary, Calgary, Canada*

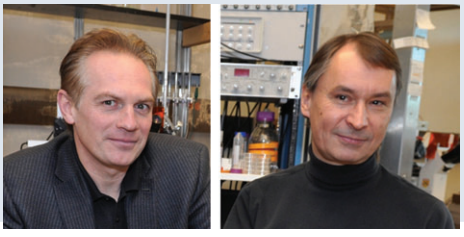
Abstract *N*-Methyl-D-aspartate (NMDA) receptors mediate a wide range of important nervous system functions. Conversely, excessive NMDA receptor activity leads to cytotoxic calcium overload and neuronal damage in a wide variety of CNS disorders. It is well established that NMDA receptors are tightly regulated by a number of cell signalling pathways. Recently, it has been shown that NMDA receptor activity is modulated by cellular prion protein (PrP^C) in a copper-dependent manner. Here we give an overview of the current state of knowledge concerning the novel concept of potent modulation of this receptor's kinetics by copper ions, and the interplay between NMDA receptors and PrP^C in the context of neurological diseases such as Alzheimer's disease, epilepsy, pain and depression.

(Received 25 November 2011; accepted after revision 3 February 2012; first published online 6 February 2012)
Corresponding author G. W. Zamponi: Department of Physiology and Pharmacology, University of Calgary, 3330 Hospital Drive NW, Calgary, Canada T2N 4N1. Email: zamponi@ucalgary.ca

NMDA receptor function

N-Methyl-D-aspartate (NMDA) receptors are one of the major classes of ionotropic glutamate receptors in the mammalian brain (McBain & Mayer, 1994). Their activation by glutamate or the synthetic agonist NMDA, together with the co-agonist glycine (or D-serine) (Shleper *et al.* 2005), results in the opening of a non-selective cation channel that mediates entry of calcium and sodium ions into the cytosol. At the synapse, glutamate release from presynaptic terminals diffuses across the synaptic cleft to activate these receptors, which in turn depolarize the postsynaptic membrane to induce an excitatory postsynaptic potential (EPSP). In addition, there are extrasynaptic NMDA receptors that are activated by glutamate spillover (Asztely *et al.* 1997; Lozovaya *et al.* 1999; Diamond, 2001). It is well established that NMDA receptors play a critical function in learning and memory (Herron *et al.* 1986; Collingridge, 1987; MacDonald *et al.* 2006). However, altered expression or function of these receptors is likely to play a role in the pathophysiology of a wide variety of CNS disorders including ischaemia, epilepsy, many neurodegenerative diseases and even neuropsychiatric disorders such as schizophrenia (Lipton & Rosenberg, 1994; Aarts *et al.* 2002; Loftis & Janowsky, 2003). The fact that NMDA receptors fulfil key physiological functions poses a dilemma with regard to designing therapeutics targeted towards these receptors, although a balance can be struck as evidenced by the Alzheimer's drug

Drs **Gerald Zamponi** and **Peter Stys** work at the University of Calgary's Hotchkiss Brain Institute. Dr Zamponi is the Head of the Department of Physiology and Pharmacology and a basic neuroscientist. His interest lies in the molecular physiology of voltage and ligand gated ion channels with an emphasis on pain and neurodegenerative disorders. Dr Stys is a neurologist and clinician scientist with expertise in multiphoton imaging techniques and a research interest in multiple sclerosis and neurodegeneration. Through a collaborative effort Drs Zamponi and Stys have discovered a new mechanism by which Aβ mediates neuronal degeneration via a copper dependent dysregulation of NMDA receptors.



This report was presented at the First International Meeting on Ion Channel Signaling Mechanisms, *Ion channel signalling mechanisms: from basic science to clinical applications*, Marrakesh, Morocco, 31 October–4 November 2011.

DOI: 10.1113/jphysiol.2011.225276

memantine, which is an NMDA receptor antagonist with relatively modest side effects (Lipton, 2006; Olivares *et al.* 2011). At negative voltages near the resting membrane potential, most NMDA receptors are tonically inhibited by extracellular magnesium ions (Nowak *et al.* 1984). Membrane depolarization dislodges magnesium from its binding site in the NMDA receptor pore, thus conferring a voltage dependence on receptor activity (Mayer *et al.* 1984). In addition to magnesium, zinc ions also bind to the receptors outside of the pore to modulate activity (Peters *et al.* 1987). As we will outline below, another metal ion that is emerging as an important NMDA receptor modulator is copper.

NMDA receptors activate (i.e. open) in response to ligand binding, and they deactivate (i.e. close) upon ligand unbinding (Vance *et al.* 2011). During prolonged application of agonist, these receptors undergo desensitization (Mayer *et al.* 1989), a process conceptually similar to voltage-dependent inactivation in voltage-gated calcium and sodium channels (Stotz *et al.* 2004), and designed to limit toxic calcium overload of cells during periods of prolonged glutamate elevations. Receptor desensitization is modulated by the aforementioned co-agonist glycine, such that increasing concentrations of this ligand produce a dramatic slowing of receptor desensitization kinetics (Mayer *et al.* 1989; Vyklicky *et al.* 1990). The physiological importance of NMDA receptor desensitization is underscored by the observation that application of high glycine concentrations is toxic to neurons, and blocking glycine reuptake increases NMDA receptor mediated neuronal excitability (Chen *et al.* 2003). Hence, receptor kinetics must be tightly controlled to maintain critical NMDA receptor functionality while preventing receptor hyperactivity and cellular damage.

NMDA receptor structure and subunit composition

NMDA receptors are heterotetramers that contain two obligatory GluN1 subunits (formerly known as NR1; Collingridge *et al.* 2009) and various combinations of GluN2A–D (formerly NR2A–D) and GluN3A–B (formerly NR3A–B) subunits (Paoletti & Neyton, 2007). Each of these subunits share a common transmembrane topology with a large extracellular N-terminal domain, and four membrane helices, as well as a large cytoplasmic C-terminal region (see Fig. 1) (Paoletti, 2011). In mammals, there is only one gene encoding GluN1, although different isoforms can be generated through alternative splicing (Tingley *et al.* 1993). The GluN1 subunit contains the binding site for the co-agonist glycine and D-serine which interact at a site formed by the N-terminal domain and the extracellular linker connecting the third and fourth transmembrane domains (for review see Paoletti, 2011). There are four different types of GluN2 subunits (GluN2A, B, C and D) which can be incorporated with the two obligatory GluN1 subunits into the assembled NMDA receptor complex, or with GluN3 to form triheteromers (Low & Wee, 2010; Rauner & Kohr, 2011). The GluN2 subunit contains the glutamate binding site, which, similarly to the GluN1 glycine binding site, is formed by interactions between the N-terminal domain and the loop between M3 and M4. The GluN2 subunit also contains the zinc interaction site which is located within the N-terminal domain (Choi & Lipton, 1999). The GluN3 subunits (i.e. GluN3A and GluN3B) do not bind glutamate, but instead interact with glycine, thus giving rise to a 'glycine-only NMDA receptor' when assembled with GluN1 in the absence of GluN2 (Chatterton *et al.* 2002; Piña-Crespo *et al.* 2010). In the context of this article, these glutamate-independent

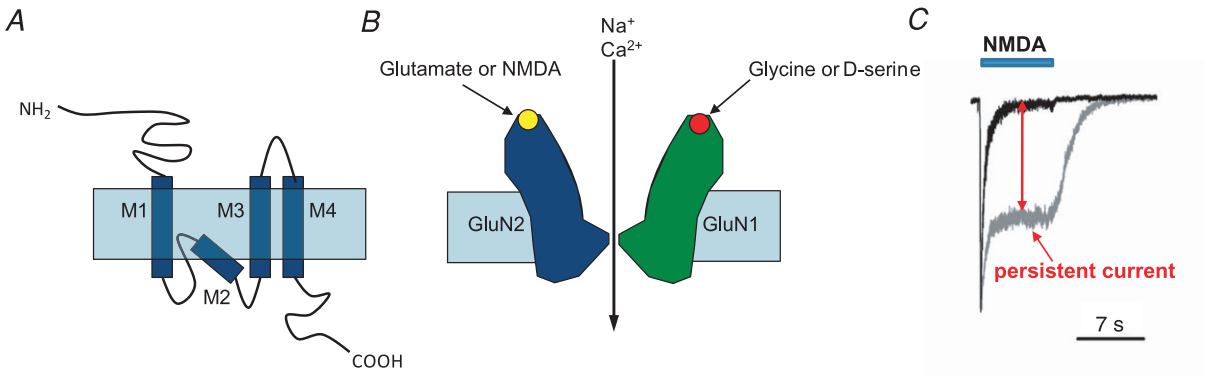


Figure 1 NMDA receptor structure and kinetics
A, transmembrane topology of a basic NMDA receptor subunit (i.e. GluN1, GluN2 or GluN3). Note the large N-terminus region that is involved in ligand binding. *B*, schematic representation of an NMDA receptor. One GluN1 and one GluN2 subunit are shown, although the fully assembled receptor complex contains two GluN1 and two GluN2 (or GluN3) subunits. *C*, effect of glycine on NMDA receptor desensitization. Two current recordings obtained from a wild-type mouse hippocampal neuron are depicted and scaled to overlap at peak. Both traces were evoked by 500 μM NMDA, the traces shown in black and grey were obtained, respectively, in the presence of 300 nM and 10 μM glycine.

receptors are not considered further. In a fully assembled receptor, the pore region is formed by residues within the M2 regions which function similarly to the p-loops found in other types of ion channels (Kupper *et al.* 1996). Specific amino acid residues in the pore region ensure selectivity for cations, and the ability to bind magnesium ions or blockers such as MK801 (Mori *et al.* 1992).

The different types of GluN2 subunits generate receptor complexes with distinct functional and pharmacological properties (Loftis & Janowsky, 2003; Cull-Candy & Leszkiewicz, 2004; Paoletti & Neyton, 2007; Paoletti, 2011). As demonstrated in heterologous expression systems, GluN2 subunits govern a number of important NMDA receptor properties, including deactivation and desensitization rates, glycine and glutamate affinity, maximal open probability and susceptibility to magnesium block (reviewed in Paoletti, 2011). Furthermore, in neurons, the subcellular distribution of the receptors is GluN2 subtype dependent with GluN2A-containing receptors being localized to synaptic sites, whereas GluN2B containing receptors are found extrasynaptically (Liu *et al.* 2007), although this arrangement is not absolute (Thomas *et al.* 2006). At least in rodents, the expression of GluN2 subunits is thought to change with age, thus adding further complexity and suggesting a specific role of GluN2 subunits at various stages of neuronal development (Loftis & Janowsky, 2003).

NMDA receptors do not operate in isolation. They are tightly regulated by second messengers such as src and fyn kinases and CamKII which act on the large cytoplasmic C-terminal domain of the receptor (Xu *et al.* 2008; Sanhueza *et al.* 2011; Trepanier *et al.* 2012). Furthermore, NMDA receptors interact with certain types of G-protein coupled receptors (such as dopamine receptors) to form macromolecular signalling complexes (Lee *et al.* 2002). Recent work from our laboratory has revealed that NMDA receptors interact with, and are regulated by, cellular prion protein (PrP^C) (Khosravani *et al.* 2008; You *et al.* 2012). Below, we will focus on the effect of PrP^C on NMDA receptor function, and its possible pathophysiological roles.

Structure and physiological function of PrP^C

Before normal cleavage, PrP^C is a 254 amino acid protein that is ubiquitously expressed and has highly conserved homologues even in simple organisms such as yeast. It contains a 22 amino acid N-terminal signal peptide, followed, depending on the species, by four to five octapeptide repeats contained within an unstructured N-terminal region (Riesner, 2003; Aguzzi & Heikenwalder, 2006). The C-terminal half of the protein contains three α-helices and a glycophosphatidylinositol (GPI) anchor at position 231 that mediates an association with the extracellular leaflet of the plasma membrane (Aguzzi & Heikenwalder, 2006). The octapeptide repeats contain up to five copper binding sites with affinities varying from the femtomolar to the micromolar range (Jackson *et al.* 2001; Burns *et al.* 2003). Conversion of PrP^C into the pathological β-sheet-rich scrapie conformation (i.e. PrP^{Sc}) has been associated with neurological disorders such as Creutzfeldt–Jakob disease (Kingsbury *et al.* 1983; Palmer *et al.* 1991; Mallucci *et al.* 1999), bovine spongiform encephalopathy (BSE) (Hill *et al.* 1997; Prusiner, 1997), and Kuru (Gajdusek, 1977). Once misfolded, PrP^{Sc} acts as a chaperone that triggers the misfolding of normal PrP^C, thus leading to a progressive accumulation of PrP^{Sc} (Aguzzi *et al.* 2008). This in turn gives rise to aggregates that may cause massive neuronal degeneration that ultimately culminate in death of the subject (Caughey *et al.* 2009). Therefore, much effort has focused on understanding the role and function of PrP^{Sc}, and the molecular and biochemical mechanisms that trigger misfolding of normal PrP^C. In contrast, the normal physiological function of PrP^C remains incompletely understood (Linden *et al.* 2008). Interestingly, copper, likely acting via the copper-binding octarepeats, induces significant conformational changes to PrP^C (Wong *et al.* 2003), which may have important implications for the regulation of NMDARs (see below). There is also accumulating evidence that PrP^C may not only play an important role in neuroprotection (Khosravani *et al.* 2008), but also act as a conduit for compromised neuronal function and ultimately degeneration in disorders not typically considered prionopathies, such as Alzheimer's disease (Lauren *et al.* 2009).

Mice lacking PrP^C display a surprisingly mild behavioural phenotype (Bueler *et al.* 1992; for review see Steele *et al.* 2007) unless subjected to an insult. Under normal circumstances, these mice reportedly show slight deficits in spatial learning (Nishida *et al.* 1997; Valenti *et al.* 2001; Criado *et al.* 2005) and at an advanced age, peripheral nerve demyelination (Bremer *et al.* 2010). Reports based on brain slice recordings suggest altered long term potentiation (Collinge *et al.* 1994; Johnston *et al.* 1998; Curtis *et al.* 2003; Rangel *et al.* 2009), consistent with these memory deficits. Notably, PrP^C null mice are resistant to infectious prions, because these mice lack the template for further conversion of PrP^C into PrP^{Sc} and accumulation of the latter (Sailer *et al.* 1994). Along these lines, knockdown of PrP^C prevents PrP^{Sc} toxicity in rodents (White *et al.* 2008; White & Mallucci, 2009). However, there is increasing evidence that the absence of PrP^C can in fact be harmful. For example, PrP^C null mice show greater mortality following chemically nduced (i.e. pentylenetetrazole and pilocarpine) seizures, and a greater incidence of seizures in the kainic acid model (Walz *et al.* 1999), although these findings have been disputed in a

more recent study (Ratte *et al.* 2011). PrP^C null mice display enhanced and prolonged neuroinflammation in a model of experimental autoimmune encephalomyelitis (EAE), whereas overexpression of PrP^C was protective in the EAE model (Tsutsui *et al.* 2008). Moreover, silencing of PrP^C has been shown to accelerate CNS autoimmune disease in a T-cell-dependent manner (Hu *et al.* 2010). Finally, recent findings from our laboratory have revealed that PrP^C null mice display depressive-like behaviour (Gadotti *et al.* 2012), and exhibit heightened nociception and increased inflammatory pain, as well as central pain sensitization (Gadotti & Zamponi, 2011). Interestingly, both of these behaviours could be abrogated by treatment with the NMDA receptor antagonist MK-801, suggesting the possibility that NMDA receptor activity may be enhanced by the absence of PrP^C. Together, these findings indicate that PrP^C may have multiple neuroprotective roles, thus perhaps explaining why this protein is so widely expressed.

PrP^C regulation of NMDA receptors

PrP^C appears to regulate the function of a wide range of different ion channels and receptors. For example, L-type calcium channel amplitude is decreased when the channels are exposed to the copper binding domain of recombinant PrP^C (Korte *et al.* 2003). Knockout of PrP^C results in a reduction of calcium activated potassium currents in cerebellar Purkinje cells (Herms *et al.* 2001), although it is not clear if this is a direct action on the potassium channel, or an indirect effect mediated via altered calcium entry. Along these lines, application of a 20 amino acid central fragment of PrP^C (residues 106–126) triggers the activation of potassium currents in basal forebrain neurons (Alier *et al.* 2010). A subsequent report revealed that PrP^C interacts with the potassium channel tetramerization domain (Huang *et al.* 2012), together indicating that PrP^C is an important regulator of various types of potassium channels. A recent study revealed that PrP^C interacts with mGLuR1 and mGluR5 receptors to stimulate ERK1/2 activation (Beraldo *et al.* 2011) suggesting that PrP^C can regulate G protein-coupled receptor-mediated cell signalling. Ligand-gated channels are also regulated by PrP^C. For example, the α7-nicotinic receptor activity is regulated by stress-inducible-protein-1 via PrP^C (Beraldo *et al.* 2010), and PrP^C is known to regulate kainate receptor expression (Rangel *et al.* 2007). Altogether these examples illustrate that PrP^C is a promiscuous protein that has the propensity to regulate signalling by plasma membrane receptors and channels (for a more detailed review, see Linden *et al.* 2008).

It may thus not be surprising to note that NMDA receptor activity is subject to modulation by PrP^C and PrP^{Sc}. Indeed, in addition to the *in vivo* data mentioned in the previous section, regulation of NMDA receptor function by PrP^C is also supported by findings that MK801 protects from increased cell death in PrP null mice subjected to kainate excitotoxicity (Rangel *et al.* 2007), presumably because the kainate-induced membrane depolarization leads to a secondary activation of NMDA receptors. Furthermore, neuronal cultures infected with PrP^{Sc} show increased survival in the presence of MK801 (Muller *et al.* 1993).

A recent study from our group examined the cellular basis for these effects (Khosravani *et al.* 2008). In field potential recordings from hippocampal slices from PrP null mice there was an increase in the number of population spikes compared with those seen in WT slices. This effect was exacerbated upon removal of magnesium ions, and normalized in the presence of the NMDA receptor blocker amino-5-phosphonovaleric acid. This hyperexcitability seen with PrP null slices fits with the increased susceptibility of PrP^C null mice to seizure-like behaviour. Miniature synaptic NMDA currents were also enhanced both in amplitude and duration, and whole cell NMDA currents in cultured pyramidal neurons showed greater current amplitude and dramatically slowed deactivation kinetics. Consistent with NMDA receptor hyperfunction, direct injection of NMDA into PrP null mouse brains resulted in dramatically increased lesion size compared to WT animals (Khosravani *et al.* 2008). The slowed deactivation kinetics appear reminiscent of what is observed with transiently expressed NMDA receptors containing the GluN2D subunit (Cull-Candy & Leszkiewicz, 2004), perhaps suggesting that the absence of PrP^C may lead to altered NMDA receptor subunit composition. However, although GluN2D siRNA treatment resulted in smaller, faster deactivating currents, it is unlikely that PrP null mouse neurons contain pure GluN1/GluN2D receptors, because whole cell currents could still be blocked by a combination of GluN2A and GluN2B blockers (H. You and G. W. Zamponi, unpublished observations). Rather, we suspect that in the absence of PrP^C, there is an increased proportion of heteromeric GluN1/GluN2(A or B)/GluN2D receptors which may give rise to altered deactivation kinetics, thus potentially reconciling our findings. The observation that synaptic NMDA currents are upregulated may explain the increased pain phenotype in PrP null mice, as NMDA receptors expressed in the dorsal horn of the spinal cord are similarly upregulated during central pain sensitization (i.e. 'wind-up') (Woolf & Thompson, 1991; Salter, 2005; Liu *et al.* 2008).

As noted above, NMDA receptor inhibitors prevent cell death of cultured neurons exposed to PrP^{Sc} (Muller *et al.* 1993). In principle, this could be explained by one of three different scenarios. First, exposure to PrP^{Sc} may cause the misfolding of endogenous PrP^C, thus leading to a *de facto* PrP^C knockdown and increased NMDA

receptor activity. Indeed, a reduction in PrP^C levels has been reported in mouse models of prion disease (Griffin *et al.* 2007). Alternatively, it is conceivable that PrP^{Sc} could directly stimulate NMDA receptor function. Finally, it is possible that PrP^{Sc} may be unable to inhibit NMDA receptor activity, thereby acting as a dominant negative inhibitor of PrP^C action on the receptor complex.

Copper modulates NMDA receptors via PrP^C

It is well established that PrP^C is a high affinity copper binding protein (Brown *et al.* 1997; Jackson *et al.* 2001; Brown & Sassoon, 2002); however the physiological role of the copper binding sites on PrP^C has been elusive. We have recently shown that chelation of copper ions potently regulates native NMDA receptors in rat and mouse hippocampal neurons (You *et al.* 2012). As noted earlier, glycine acts as a potent co-agonist at the GluN1 receptor and a negative regulator of NMDA receptor desensitization. Many investigators in the NMDA receptor field conduct recordings in high concentrations of glycine as an experimental manipulation to boost current responses. Interestingly, both glycine and D-serine chelate copper ions (Martin *et al.* 1971), therefore the balance between applied agonist concentration and ambient copper levels becomes critically important. For this reason, we conducted our experiments using a physiological glycine concentration that is thought to reflect the submicromolar levels normally present at the synapse (Supplisson & Roux, 2002; Yang & Svensson, 2008). When neurons were exposed to 300 nM glycine/500 μM NMDA, currents in cultured pyramidal cells desensitized almost completely (Fig. 1*C*, black trace). Exposure of neurons to selective copper chelators such as bathocuproine sulfonate (BCS) or cuprizone induced a persistent non-desensitizing current component that could be overcome by addition of excess copper ions. Interestingly, in 300 nM glycine (without copper chelation), neurons from PrP null mice behaved exactly like BCS treated WT neurons; moreover the effects of PrP^C knockout and BCS were not additive, suggesting that the effects of copper on NMDA receptor desensitization were mediated via PrP^C. Furthermore, PrP^C could be immunoprecipitated with GluN1, and the strength of this interaction was greatly weakened upon copper chelation. This, together with the observation that the glycine affinity for the receptor was enhanced in PrP^C null mice (or upon application of BCS) led us to propose a model in which copper-dependent allosteric interactions between PrP^C and the GluN1 subunit regulate the affinity of the receptor complex for the co-agonist glycine, in this manner leading to non-desensitizing currents at any given glycine concentration (Fig. 2). This then suggests that perhaps a key physiological role of the copper binding sites on PrP^C is to regulate NMDA receptors. However, even in the absence of PrP^C, micromolar concentrations of copper still speed NMDA receptor desensitization (You *et al.* 2012; see also Vlachova *et al.* 1996), indicating the presence of a second copper modulation mechanism that operates independently of PrP^C. One possibility may be an as-yet-unidentified copper binding site on the receptor, as was discovered for zinc (Rachline *et al.* 2005). The intrinsic mechanisms that give rise to persistent currents in NMDA receptors upon copper chelation/deletion of PrP^C are not known. One attractive possibility is a switch in modal gating of the receptor, as described recently in a study by Zhang *et al.* (2008). It is also interesting to note that GluN2D-containing receptors show little desensitization and display the highest glycine affinity of all NMDA receptor subtypes (Erreger *et al.* 2007; Chen *et al.* 2008). However, given that the effects of copper chelation occurred rapidly, a switch in NMDA receptor subunit composition is unlikely to account for the persistent currents.

The regulation of NMDA receptors by copper ions deserves additional discussion. First, BCS might be a selective chelator of Cu^+ over Cu^{2+} whereas PrP^C is thought to specifically bind Cu^{2+}. However, the interactions between BCS and the two oxidation states of copper are non-trivial, with BCS able to bind Cu^{2+} with high affinity to form bis $[Cu(BCS)_2]^{2-}$ complexes (Al-Shatti *et al.* 1981; Sayre, 1996) or the protonated form of BCS $[H{\cdot}BCS]^-$ associates with Cu^{2+} to form complex species such as $[Cu^{2+}\ (BCS)\ (H_2O)_x]$ and a proton (Xiao *et al.* 2011); in a pH buffer, this proton will be buffered driving the reaction in favour of complete chelation of all Cu^{2+} by BCS (or more precisely, by its mono-protonated form). The fact that the canonical Cu^{2+}-selective chelator cuprizone (Peterson & Bollier, 1955) yielded identical results supports the idea that BCS indeed acted as a high-affinity copper chelator independent of this metal's oxidation state.

There is a large body of literature on the copper concentrations in CSF, with published values ranging widely from 0.12 to 7 μM (Kanabrocki *et al.* 1964; Agarwal & Henkin, 1982). In the whole brain, levels are around 5 μg (g wet weight)$^{-1}$ ($\sim$80 μM equivalent) (Warren *et al.* 1960). The problem with determining CSF or whole brain copper levels is that almost all of this metal is bound to proteins and amino acids; thus the copper available for binding to PrP^C is determined not so much by the measured total concentrations, but rather by the relative affinities of PrP^C vis-à-vis those of other copper binding species. Measured copper in our culture media was around 100 nM although other media range up to the low micromolar level. The resting copper concentration in the synaptic cleft where the NMDA receptors are located is also estimated to be $\sim$1 μM, though transient peak levels may approach 250 μM (reviewed in Millhauser, 2007). Given the ability of copper to

potently modulate NMDA receptor kinetics and limit agonist-induced inward current and therefore Ca^{2+} loads (see above), it is plausible that ambient copper in culture media and brain extracellular space functions to limit NMDA receptor-dependent excitotoxicity. In line with this prediction, treatment of cultured neurons with copper chelators causes cell death that can be prevented by the NMDA receptor antagonist 5,7-dichlorokynurenic acid or by supplying excess copper (You *et al.* 2012), underscoring the potential key importance of trace copper in cell culture media and experimental perfusing solutions. Neurons have active copper transport mechanisms such as the copper ATPases (Niciu *et al.* 2006; Veldhuis *et al.* 2009) that, along with copper binding proteins such as PrP^C, ensure proper copper homeostasis in the brain. When cultured neurons or brain slices are placed in recording chambers filled with external solutions such as aCSF (which according to our measurements contain anywhere between 4 nM and 50 nM total copper) for prolonged periods of time, one might expect a time-dependent partial depletion of copper to occur. As noted earlier, PrP^C contains several copper binding sites whose affinities vary widely from the micromolar to the femtomolar range (Jackson *et al.* 2001). In media or perfusing solutions containing sub-physiological concentrations of copper (e.g. in the tens of nanomolar, compare with published CSF concentrations noted above), it is conceivable that the lower affinity copper-binding sites on PrP^C will be depleted of this metal, potentially significantly altering the physiology or even the survival of the neuron. On the other hand it is important to note that prolonged exposure of neurons to higher levels of copper is also toxic due to the generation of free radicals (Simpson *et al.* 1988). This may explain findings from Sassoon *et al.* (2004) showing that prolonged exposure of neurons to 10 μM copper exacerbated the effects of a toxic PrP^C fragment.

In our experiments, appearance of persistent currents upon application of BCS occurred with a lag of approximately 2 min, consistent with a mechanism whereby PrP^C is initially loaded with copper, and BCS (which binds copper with attomolar affinity) slowly

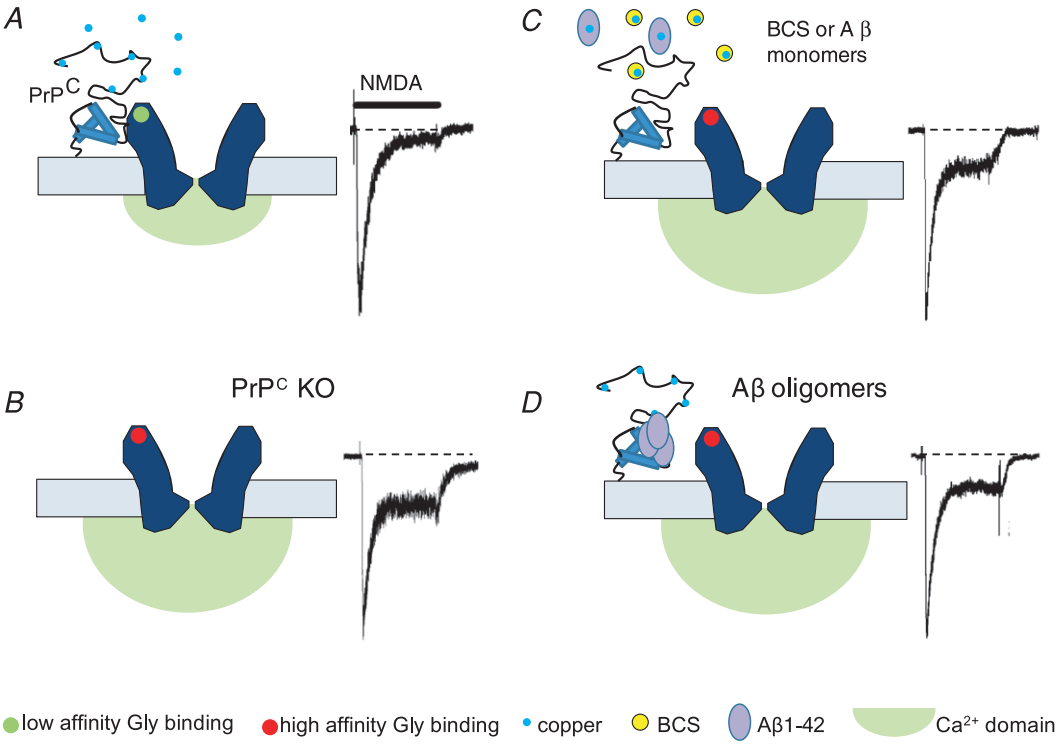


Figure 2. Model depicting the effects of copper and PrP^C on NMDA receptor function
A, under normal circumstances, PrP^C in its copper-loaded state exists in a complex with the NMDA receptor, reducing the affinity of the co-agonist glycine for the receptor. At physiological levels of glycine (1 μM or lower), this results in strong desensitization of the receptor (see current trace obtained from a cultured hippocampal neuron). *B*, knockout of the gene encoding PrP^C results in a receptor with higher affinity for glycine, thus leading to persistent non-desensitizing currents and overall greater accumulation of calcium in the cell. *C*, chelation of copper ions by the selective chelator bathocuproine sulfonate or by A*β monomers* causes a weakening of the association between PrP^C and the NMDA receptor, thus producing currents similar to those seen in PrP^C null mouse neurons. *D*, A*β oligomers* bind directly to PrP^C to disrupt PrP^C regulation of NMDA receptor activity, producing the same phenotype as that shown in panels *B* and *C*.

chelates copper ions that unbind from PrP^C. This suggests that copper ions are likely to be bound to PrP^C (at least at the higher affinity sites) even when bathed in aCSF, either from trace copper in the solutions or from copper ions released by the neurons themselves, or because copper ions may be leaching from the experimental apparatus. Altogether, these considerations suggest that maintenance of consistent copper homeostasis during experiments is critical. By extension, it is conceivable that some of the variability in the extensive published literature pertaining to NMDA receptor physiology could be attributed to variable trace copper levels in media and perfusing fluids. We would therefore argue that copper ions might have to be supplied exogenously to maintain consistent concentrations, similar to what is done routinely with magnesium.

It should be noted that copper ions not only modulate NMDA receptors, but also a variety of other types of ion channels. For example, AMPA receptors in rat cortical neurons are blocked with IC_{50} values of around 5 μM, and at saturating levels of copper (i.e. 30 μM) agonist affinity for these receptors is reduced (Weiser & Wienrich, 1996). Copper ions also block T-type calcium channels and high voltage activated calcium channels with affinities ranging from ~1 to 30 μM depending on calcium channel subtype (Jeong *et al.* 2003; Lu *et al.* 2009), and they modulate ENaC channels in the submicromolar range (Lu *et al.* 2009). These blocking affinities are similar to those observed in our own experiments on NMDA receptors in the absence of PrP^C, which fits with the idea that voltage gated cation channels may show common architectural features in their pore forming regions – the presumed site of action of copper. In the nanomolar range, copper ions have been shown to enhance the firing of olfactory epithelium neurons, whereas concentrations above 1 μM were inhibitory (Aedo *et al.* 2007). These potent effects of copper on ion channel physiology and neuronal activity further underscore the need to clamp copper concentrations at a physiological level during *in vitro* studies of the nervous system.

Aβ-mediated regulation of NMDA receptor function

NMDA receptors have been implicated in Alzheimer's disease (AD), and the NMDA receptor blocker memantine is Food and Drug Administration (FDA) approved for the treatment of this disorder (Kalia *et al.* 2008; Kotermanski & Johnson, 2009; Di Stefano *et al.* 2011). One of the key aspects of AD is an overproduction of toxic Aβ_{1-42} peptides in the brain. Application of Aβ_{1-42} oligomers to cultured neurons has been shown to trigger spontaneous NMDA currents and longer term exposure of neurons to Aβ_{1-42} leads to NMDA receptor internalization (Snyder *et al.* 2005; Texido *et al.* 2011). It was recently reported by Lauren *et al.* (2009) that Aβ_{1-42} oligomers can interact with PrP^C and that the toxic effect of Aβ_{1-42} is dependent on these interactions. Furthermore, Aβ_{1-42} is a high affinity copper binding peptide (Atwood *et al.* 2000), with values similar to those reported for BCS. Altogether, these considerations suggest a possible link between Aβ_{1-42}, copper, PrP^C and NMDA receptors. Indeed, as we have shown recently (You *et al.* 2012), application of nanomolar concentrations of Aβ_{1-42} oligomers, or micromolar concentrations of Aβ_{1-42} monomers, produced effects on NMDA receptors that were indistinguishable from those of BCS or removal of PrP^C (either acutely via GPI anchor cleavage or by gene knockout). Furthermore, recordings from neurons cultured from hemizygous 5XFAD mice, a mouse model of Alzheimer's disease where large amounts of Aβ_{1-42} are produced within neurons (Oakley *et al.* 2006), revealed more slowly desensitizing NMDA currents compared to wild-type littermates, again with characteristics very similar to those observed with BCS treatment or PrP^C ablation. Like BCS, application of Aβ_{1-42} to cultured neurons causes cell death that could be prevented either with NMDA receptor antagonists, or by addition of excess copper (You *et al.* 2012). Together these data suggest that an interplay between copper, Aβ_{1-42}, PrP^C and NMDA receptors may play a pivotal role in AD pathogenesis. We envision that high affinity binding of oligomers directly to PrP^C mediates an allosteric inhibition of NMDA receptor desensitization (similar to the absence of PrP^C), whereas higher concentrations of monomer might act simply by copper chelation (similar to BCS). Therefore, Aβ peptides are likely to mediate their deleterious effects by two related, but distinct mechanisms: one dependent on PrP^C where oligomers interfere with normal physiological function (i.e. regulation of NMDA receptor kinetics) of this ubiquitous cuproprotein, and the second by more directly interfering with the ability of copper to modulate these receptors. Such a dual mode of Aβ action would elegantly reconcile the ongoing controversy surrounding the requirement of PrP^C for the observed pathological effects of this peptide (Lauren *et al.* 2009; Calella *et al.* 2010; Gimbel *et al.* 2010; Kessels *et al.* 2010; Freir *et al.* 2011) (see below).

If elimination of PrP^C mediates similar electrophysiological consequences compared to those of Aβ_{1-42}, why do PrP null mice not exhibit synaptic and neuronal degeneration as in AD? There are at least two key differences between the absence of PrP^C and the accumulation of Aβ_{1-42}. First, a slowing of NMDA receptor desensitization only matters under conditions where there is prolonged exposure to (i.e. excess) glutamate. Indeed, Aβ_{1-42} is known to reduce glutamate reuptake in hippocampal neurons (Li *et al.* 2009), which would lead to increased glutamate concentrations and toxic persistent currents that would not be observed in PrP null mice. Second, as noted earlier, NMDA receptors

can be blocked by copper independently of PrP^C. Given that higher copper concentrations can compensate for the absence of PrP^C by restoring NMDA receptor kinetics to near normalcy, it is possible that in the PrP null brain, compensatory mechanisms are able to maintain free copper in key locations (e.g. in the synaptic cleft) at higher levels to mitigate the adverse influence of a PrP^C deficit on this key receptor.

It is important to note that the involvement of PrP^C in the toxic effects of $A\beta_{1-42}$ have become somewhat controversial. In support of the finding of Lauren and colleagues, Barry *et al.* (2011) reported that suppression of *in vivo* long-term potentiation by $A\beta_{1-42}$ oligomers was dependent on PrP^C. Freir *et al.* (2011) reported that $A\beta_{1-42}$ isolated from human AD brains could alter neuronal plasticity in a manner dependent on PrP^C. Finally, Gimbel *et al.* (2010) reported that deletion of PrP^C could abrogate the memory impairment seen in a mouse AD model. However, several other groups were unable to confirm a PrP^C dependence of the effects of $A\beta_{1-42}$ on synaptic physiology (Calella *et al.* 2010; Kessels *et al.* 2010). It is possible that different levels of copper and glycine used in these various studies lie at the root of these discordant findings, again stressing the need to maintain copper at a known fixed concentration in neurophysiological studies involving NMDA receptors.

Another perplexing question that arises is, why is PrP^{Sc} so toxic to neurons? If complete absence of PrP^C, by our arguments a very important NMDA receptor regulator, results in minimal neuronal toxicity (perhaps because ambient copper levels are adjusted accordingly), what is the mechanism of such extreme toxicity of some species of scrapie? The answer is not known, but it is conceivable that prion protein may assume many different conformation states, with PrP^{Sc} being the most nefarious. In this conformation, it is possible that the modulatory influence on NMDA receptors is so disadvantageous, potentially inducing high persistent currents that cannot be rescued by any compensatory mechanisms that are invoked in tissue merely lacking PrP. As a result of neurons being locked into a 'chronic excitotoxic state', massive degeneration occurs, which has been shown to be at least partially NMDA receptor dependent (Muller *et al.* 1993; Schroder *et al.* 1998). Clearly, this hypothesis will have to be tested experimentally.

Concluding remarks

Copper ions appear to mediate potent regulation of NMDA receptors akin to what has been described for zinc nearly two decades ago. Dysregulation of copper homeostasis near the NMDA receptor can trigger aberrant, slowly desensitizing currents that result in toxic calcium overload in the presence of sustained glutamate levels. This in turn may contribute to the pathology of Alzheimer's disease. Copper dysregulation of NMDA receptors may also play a role in other neurological disorders. For example, in Menkes disease, a mutation in the copper ATPase mediates NMDA receptor-dependent neurodegeneration (Schlief *et al.* 2005, 2006; Schlief & Gitlin, 2006). Furthermore, both α-synuclein and huntingtin are known to be copper binding proteins (Fox *et al.* 2007; Davies *et al.* 2010), and both have been associated with NMDA receptor mediated neuronal toxicity (Fan & Raymond, 2007; Adamczyk *et al.* 2009). This raises the possibility of copper modulation of NMDA receptors (perhaps via PrP^C) as a unifying theme in many neurodegenerative disorders.

References

Aarts M *et al.* (2002). Treatment of ischemic brain damage by perturbing NMDA receptor- PSD-95 protein interactions. *Science* **298**, 846–850.

Adamczyk A, Czapski GA, Kazmierczak A & Strosznajder JB (2009). Effect of N-methyl-D-aspartate (NMDA) receptor antagonists on α-synuclein-evoked neuronal nitric oxide synthase activation in the rat brain. *Pharmacol Rep* **61**, 1078–1085.

Aedo F, Delgado R, Wolff D & Vergara C (2007). Copper and zinc as modulators of neuronal excitability in a physiologically significant concentration range. *Neurochem Int* **50**, 591–600.

Agarwal RP & Henkin RI (1982). Zinc and copper in human cerebrospinal fluid. *Biol Trace Element Res* **4**, 117–124.

Aguzzi A & Heikenwalder M (2006). Pathogenesis of prion diseases: current status and future outlook. *Nat Rev Microbiol* **4**, 765–775.

Aguzzi A, Sigurdson C & Heikenwaelder M (2008). Molecular mechanisms of prion pathogenesis. *Annu Rev Pathol* **3**, 11–40.

Al-Shatti N, Lappin AG & Sykes AG (1981). Influence of coordination number on copper(I)-Copper(II) redox interconversions. 2. Fe(CN)64- reduction of a sterically constrained bis(substituted phenanthroline) complex of copper(II) in aqueous solution. *Inorg Chem* **20**, 1466–1469.

Alier K, Li Z, Mactavish D, Westaway D & Jhamandas JH (2010). Ionic mechanisms of action of prion protein fragment PrP(106–126) in rat basal forebrain neurons. *J Neurosci Res* **88**, 2217–2227.

Asztely F, Erdemli G & Kullmann DM (1997). Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. *Neuron* **18**, 281–293.

Atwood CS *et al.* (2000). Characterization of copper interactions with Alzheimer amyloid beta peptides: identification of an attomolar-affinity copper binding site on amyloid β1–42. *J Neurochem* **75**, 1219–1233.

Barry AE *et al.* (2011). Alzheimer's disease brain-derived amyloid-β-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. *J Neurosci* **31**, 7259–7263.

Beraldo FH *et al.* (2011). Metabotropic glutamate receptors transduce signals for neurite outgrowth after binding of the prion protein to laminin γ1 chain. *FASEB J* **25**, 265–279.
Beraldo FH *et al.* (2010). Role of α7 nicotinic acetylcholine receptor in calcium signaling induced by prion protein interaction with stress-inducible protein 1. *J Biol Chem* **285**, 36542–36550.
Bremer J *et al.* (2010). Axonal prion protein is required for peripheral myelin maintenance. *Nat Neurosci* **13**, 310–318.
Brown DR *et al.* (1997). The cellular prion protein binds copper in vivo. *Nature* **390**, 684–687.
Brown DR & Sassoon J (2002). Copper-dependent functions for the prion protein. *Mol Biotechnol* **22**, 165–178.
Bueler H *et al.* (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**, 577–582.
Burns CS *et al.* (2003). Copper coordination in the full-length, recombinant prion protein. *Biochemistry* **42**, 6794–6803.
Calella AM *et al.* (2010). Prion protein and Aβ-related synaptic toxicity impairment. *EMBO Mol Med* **2**, 306–314.
Caughey B, Baron GS, Chesebro B & Jeffrey M (2009). Getting a grip on prions: oligomers, amyloids, and pathological membrane interactions. *Annu Rev Biochem* **78**, 177–204.
Chatterton JE *et al.* (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* **415**, 793–798.
Chen L, Muhlhauser M & Yang CR (2003). Glycine tranporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. *J Neurophysiol* **89**, 691–703.
Chen PE *et al.* (2008). Modulation of glycine potency in rat recombinant NMDA receptors containing chimeric NR2A/2D subunits expressed in *Xenopus laevis* oocytes. *J Physiol* **586**, 227–245.
Choi YB & Lipton SA (1999). Identification and mechanism of action of two histidine residues underlying high-affinity Zn^{2+} inhibition of the NMDA receptor. *Neuron* **23**, 171–180.
Collinge J *et al.* (1994). Prion protein is necessary for normal synaptic function. *Nature* **370**, 295–297.
Collingridge G (1987). Synaptic plasticity. The role of NMDA receptors in learning and memory. *Nature* **330**, 604–605.
Collingridge GL, Olsen RW, Peters J & Spedding M (2009). A nomenclature for ligand-gated ion channels. *Neuropharmacology* **56**, 2–5.
Criado JR *et al.* (2005). Mice devoid of prion protein have cognitive deficits that are rescued by reconstitution of PrP in neurons. *Neurobiol Dis* **19**, 255–265.
Cull-Candy SG & Leszkiewicz DN (2004). Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* **2004**, re16.
Curtis J, Errington M, Bliss T, Voss K & MacLeod N (2003). Age-dependent loss of PTP and LTP in the hippocampus of PrP-null mice. *Neurobiol Dis* **13**, 55–62.
Davies P *et al.* (2010). The synucleins are a family of redox-active copper binding proteins. *Biochemistry* **50**, 37–47
Di Stefano A, Iannitelli A, Laserra S & Sozio P (2011). Drug delivery strategies for Alzheimer's disease treatment. *Expert Opin Drug Deliv* **8**, 581–603.
Diamond JS (2001). Neuronal glutamate transporters limit activation of NMDA receptors by neurotransmitter spillover on CA1 pyramidal cells. *J Neurosci* **21**, 8328–8338.
Erreger K *et al.* (2007). Subunit-specific agonist activity at NR2A-, NR2B-, NR2C-, and NR2D-containing N-methyl-D-aspartate glutamate receptors. *Mol Pharmacol* **72**, 907–920.
Fan MM & Raymond LA (2007). N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog Neurobiol* **81**, 272–293.
Fox JH *et al.* (2007). Mechanisms of copper ion mediated Huntington's disease progression. *PLoS One* **2**, e334.
Freir DB *et al.* (2011). Interaction between prion protein and toxic amyloid β assemblies can be therapeutically targeted at multiple sites. *Nat Commun* **2**, 336.
Gadotti VM, Bonfield SP & Zamponi GW (2012). Depressive-like behaviour of mice lacking cellular prion protein. *Behav Brain Res* **227**, 319–323.
Gadotti VM & Zamponi GW (2011). Cellular prion protein protects from inflammatory and neuropathic pain. *Mol Pain* **7**, 59.
Gajdusek DC (1977). Unconventional viruses and the origin and disappearance of kuru. *Science* **197**, 943–960.
Gimbel DA *et al.* (2010). Memory impairment in transgenic Alzheimer mice requires cellular prion protein. *J Neurosci* **30**, 6367–6374.
Griffin JK, Terry LA, Jackman R, Yousefi M & Cashman NR (2007). Decreased cell surface prion protein in mouse models of prion disease. *Neuroreport* **18**, 1–6.
Herms JW, Tings T, Dunker S & Kretzschmar HA (2001). Prion protein affects Ca^{2+}-activated K^+ currents in cerebellar purkinje cells. *Neurobiol Dis* **8**, 324–330.
Herron CE, Lester RA, Coan EJ & Collingridge GL (1986). Frequency-dependent involvement of NMDA receptors in the hippocampus: a novel synaptic mechanism. *Nature* **322**, 265–268.
Hill AF *et al.* (1997). The same prion strain causes vCJD and BSE. *Nature* **389**, 448–50, 526.
Hu W *et al.* (2010). Pharmacological prion protein silencing accelerates central nervous system autoimmune disease via T cell receptor signalling. *Brain* **133**, 375–388.
Huang T *et al.* (2012). PrP(C) interacts with potassium channel tetramerization domain containing 1 (KCTD1) protein through the PrP(51–136) region containing octapeptide repeats. *Biochem Biophys Res Commun* **417**, 182–186.
Jackson GS *et al.* (2001). Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci U S A* **98**, 8531–8535.
Jeong SW, Park BG, Park JY, Lee JW & Lee JH (2003). Divalent metals differentially block cloned T-type calcium channels. *Neuroreport* **14**, 1537–1540.
Johnston AR, Fraser JR, Jeffrey M & MacLeod N (1998). Synaptic plasticity in the CA1 area of the hippocampus of scrapie-infected mice. *Neurobiol Dis* **5**, 188–195.
Kalia LV, Kalia SK & Salter MW (2008). NMDA receptors in clinical neurology: excitatory times ahead. *Lancet Neurol* **7**, 742–755.

Kanabrocki EL, Case LF, Miller EB, Kaplan E & Oester YT (1964). A study of human cerebrospinal fluid: copper and manganese. *J Nucl Med* **5**, 643–648.

Kessels HW, Nguyen LN, Nabavi S & Malinow R (2010). The prion protein as a receptor for amyloid-β. *Nature* **466**, E3–5.

Khosravani H *et al.* (2008). Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. *J Cell Biol* **181**, 551–565.

Kingsbury DT, Kasper KC, Stites DP, Watson JD, Hogan RN & Prusiner SB (1983). Genetic control of scrapie and Creutzfeldt-Jakob disease in mice. *J Immunol* **131**, 491–496.

Korte S, Vassallo N, Kramer ML, Kretzschmar HA & Herms J (2003). Modulation of L-type voltage-gated calcium channels by recombinant prion protein. *J Neurochem* **87**, 1037–1042.

Kotermanski SE & Johnson JW (2009). Mg^{2+} imparts NMDA receptor subtype selectivity to the Alzheimer's drug memantine. *J Neurosci* **29**, 2774–2779.

Kupper J, Ascher P & Neyton J (1996). Probing the pore region of recombinant N-methyl-D-aspartate channels using external and internal magnesium block. *Proc Natl Acad Sci U S A* **93**, 8648–8653.

Lauren J, Gimbel DA, Nygaard HB, Gilbert JW & Strittmatter SM (2009). Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. *Nature* **457**, 1128–1132.

Lee FJ *et al.* (2002). Dual regulation of NMDA receptor functions by direct protein-protein interactions with the dopamine D1 receptor. *Cell* **111**, 219–230.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM & Selkoe D (2009). Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron* **62**, 788–801.

Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I & Brentani RR (2008). Physiology of the prion protein. *Physiol Rev* **88**, 673–728.

Lipton SA & Rosenberg PA (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *N Engl J Med* **330**, 613–622.

Lipton SA (2006). Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov* **5**, 160–170.

Liu XJ *et al.* (2008). Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. *Nat Med* **14**, 1325–1332.

Liu Y *et al.* (2007). NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J Neurosci* **27**, 2846–2857.

Loftis JM & Janowsky A (2003). The N-methyl-D-aspartate receptor subunit NR2B: localization, functional properties, regulation, and clinical implications. *Pharmacol Ther* **97**, 55–85.

Low CM & Wee KS (2010). New insights into the not-so-new NR3 subunits of N-methyl-D-aspartate receptor: localization, structure, and function. *Mol Pharmacol* **78**, 1–11.

Lozovaya NA, Kopanitsa MV, Boychuk YA & Krishtal OA (1999). Enhancement of glutamate release uncovers spillover-mediated transmission by N-methyl-D-aspartate receptors in the rat hippocampus. *Neuroscience* **91**, 1321–1330.

Lu L *et al.* (2009). Effects of copper on T-type Ca^{2+} channels in mouse spermatogenic cells. *J Membr Biol* **227**, 87–94.

MacDonald JF, Jackson MF & Beazely MA (2006). Hippocampal long-term synaptic plasticity and signal amplification of NMDA receptors. *Crit Rev Neurobiol* **18**, 71–84.

Mallucci GR *et al.* (1999). Inherited prion disease with an alanine to valine mutation at codon 117 in the prion protein gene. *Brain* **122**, 1823–1837.

Martin RP, Mosoni L & Sarkar B (1971). Ternary coordination complexes between glycine, copper (II), and glycine peptides in aqueous solution. *J Biol Chem* **246**, 5944–5951.

Mayer ML, Westbrook GL & Guthrie PB (1984). Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* **309**, 261–263.

Mayer ML, Vyklicky LJ & Clements J (1989). Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. *Nature* **338**, 425–427.

McBain CJ & Mayer ML (1994). N-methyl-D-aspartic acid receptor structure and function. *Physiol Rev* **74**, 723–760.

Millhauser GL (2007). Copper and the prion protein: methods, structures, function, and disease. *Annu Rev Phys Chem* **58**, 299–320.

Mori H, Masaki H, Yamakura T & Mishina M (1992). Identification by mutagenesis of a Mg^{2+}-block site of the NMDA receptor channel. *Nature* **358**, 673–675.

Muller WE *et al.* (1993). Cytoprotective effect of NMDA receptor antagonists on prion protein (PrionSc)-induced toxicity in rat cortical cell cultures. *Eur J Pharmacol* **246**, 261–267.

Niciu MJ, Ma XM, El Meskini R, Ronnett GV, Mains RE & Eipper BA (2006). Developmental changes in the expression of ATP7A during a critical period in postnatal neurodevelopment. *Neuroscience* **139**, 947–964.

Nishida N *et al.* (1997). Prion protein is necessary for latent learning and long-term memory retention. *Cell Mol Neurobiol* **17**, 537–545.

Nowak L, Bregestovski P, Ascher P, Herbet A & Prochiantz A (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462–465.

Oakley H *et al.* (2006). Intraneuronal β-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* **26**, 10129–10140.

Olivares D *et al.* (2011). N-Methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. *Curr Alzheimer Res* (in press)

Palmer MS, Dryden AJ, Hughes JT & Collinge J (1991). Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* **352**, 340–342.

Paoletti P (2011). Molecular basis of NMDA receptor functional diversity. *Eur J Neurosci* **33**, 1351–1365.

Paoletti P & Neyton J (2007). NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol* **7**, 39–47.

Peters S, Koh J & Choi DW (1987). Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science* **236**, 589–593.

Peterson RE & Bollier ME (1955). Spectrophotometric determination of serum copper with biscyclohexanoneoxalyldihydrazone. *Anal Chem* **27**, 1195–1197.

Piña-Crespo JC *et al.* (2010). Excitatory glycine responses of CNS myelin mediated by NR1/NR3 'NMDA' receptor subunits. *J Neurosci* **30**, 11501–11505.

Prusiner SB (1997). Prion diseases and the BSE crisis. *Science* **278**, 245–251.

Rachline J, Perin-Dureau F, Le Goff A, Neyton J & Paoletti P (2005). The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. *J Neurosci* **25**, 308–317.

Rangel A, Burgaya F, Gavin R, Soriano E, Aguzzi A & Del Rio JA (2007). Enhanced susceptibility of Prnp-deficient mice to kainate-induced seizures, neuronal apoptosis, and death: Role of AMPA/kainate receptors. *J Neurosci Res* **85**, 2741–2755.

Rangel A *et al.* (2009). Regulation of $GABA_A$ and glutamate receptor expression, synaptic facilitation and long-term potentiation in the hippocampus of prion mutant mice. *PLoS One* **4**, e7592.

Ratte S *et al.* (2011). Threshold for epileptiform activity is elevated in prion knockout mice. *Neuroscience* **179**, 56–61.

Rauner C & Kohr G (2011). Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. *J Biol Chem* **286**, 7558–7566.

Riesner D (2003). Biochemistry and structure of PrP^C and PrP^{Sc}. *Br Med Bull* **66**, 21–33.

Sailer A, Bueler H, Fischer M, Aguzzi A & Weissmann C (1994). No propagation of prions in mice devoid of PrP. *Cell* **77**, 967–968.

Salter MW (2005). Cellular signalling pathways of spinal pain neuroplasticity as targets for analgesic development. *Curr Top Med Chem* **5**, 557–567.

Sanhueza M *et al.* (2011). Role of the CaMKII/NMDA receptor complex in the maintenance of synaptic strength. *J Neurosci* **31**, 9170–9178.

Sassoon J, Daniels M & Brown DR (2004). Astrocytic regulation of NMDA receptor subunit composition modulates the toxicity of prion peptide PrP106–126. *Mol Cell Neurosci* **25**, 181–191.

Sayre LM (1996). Alzheimer's precursor protein and the use of bathocuproine for determining reduction of copper(II). *Science* **274**, 1933–1934.

Schlief ML, Craig AM & Gitlin JD (2005). NMDA receptor activation mediates copper homeostasis in hippocampal neurons. *J Neurosci* **25**, 239–246.

Schlief ML & Gitlin JD (2006). Copper homeostasis in the CNS: a novel link between the NMDA receptor and copper homeostasis in the hippocampus. *Mol Neurobiol* **33**, 81–90.

Schlief ML, West T, Craig AM, Holtzman DM & Gitlin JD (2006). Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. *Proc Natl Acad Sci U S A* **103**, 14919–14924.

Schroder HC, Perovic S, Kavsan V, Ushijima H & Muller WE (1998). Mechanisms of prionSc- and HIV-1 gp120 induced neuronal cell death. *Neurotoxicology* **19**, 683–688.

Shleper M, Kartvelishvily E & Wolosker H (2005). D-Serine is the dominant endogenous coagonist for NMDA receptor neurotoxicity in organotypic hippocampal slices. *J Neurosci* **25**, 9413–9417.

Simpson JA, Cheeseman KH, Smith SE & Dean RT (1988). Free-radical generation by copper ions and hydrogen peroxide. Stimulation by Hepes buffer. *Biochem J* **254**, 519–523.

Snyder EM *et al.* (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* **8**, 1051–1058.

Steele AD, Lindquist S & Aguzzi A (2007). The prion protein knockout mouse: a phenotype under challenge. *Prion* **1**, 83–93.

Stotz SC, Jarvis SE & Zamponi GW (2004). Functional roles of cytoplasmic loops and pore lining transmembrane helices in the voltage-dependent inactivation of HVA calcium channels. *J Physiol* **554**, 263–273.

Supplisson S & Roux MJ (2002). Why glycine transporters have different stoichiometries. *FEBS Lett* **529**, 93–101.

Texido L, Martin-Satue M, Alberdi E, Solsona C & Matute C (2011). Amyloid beta peptide oligomers directly activate NMDA receptors. *Cell Calcium* **49**, 184–190.

Thomas CG, Miller AJ & Westbrook GL (2006). Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. *J Neurophysiol* **95**, 1727–1734.

Tingley WG, Roche KW, Thompson AK & Huganir RL (1993). Regulation of NMDA receptor phosphorylation by alternative splicing of the C-terminal domain. *Nature* **364**, 70–73.

Trepanier CH, Jackson MF & Macdonald JF (2012). Regulation of NMDA receptors by the tyrosine kinase Fyn. *FEBS J* **279**, 12–19

Tsutsui S, Hahn JN, Johnson TA, Ali Z & Jirik FR (2008). Absence of the cellular prion protein exacerbates and prolongs neuroinflammation in experimental autoimmune encephalomyelitis. *Am J Pathol* **173**, 1029–1041.

Valenti P, Cozzio A, Nishida N, Wolfer DP, Sakaguchi S & Lipp HP (2001). Similar target, different effects: late-onset ataxia and spatial learning in prion protein-deficient mouse lines. *Neurogenetics* **3**, 173–184.

Vance KM, Simorowski N, Traynelis SF & Furukawa H (2011). Ligand-specific deactivation time course of GluN1/GluN2D NMDA receptors. *Nat Commun* **2**, 294.

Veldhuis NA, Gaeth AP, Pearson RB, Gabriel K & Camakaris J (2009). The multi-layered regulation of copper translocating P-type ATPases. *Biometals* **22**, 177–190.

Vlachova V, Zemkova H & Vyklicky LJ (1996). Copper modulation of NMDA responses in mouse and rat cultured hippocampal neurons. *Eur J Neurosci* **8**, 2257–2264.

Vyklicky LJ, Benveniste M & Mayer ML (1990). Modulation of N-methyl-D-aspartic acid receptor desensitization by glycine in mouse cultured hippocampal neurones. *J Physiol* **428**, 313–331.

Walz R *et al.* (1999). Increased sensitivity to seizures in mice lacking cellular prion protein. *Epilepsia* **40**, 1679–1682.

Warren PJ, Earl CJ & Thompson RH (1960). The distribution of copper in human brain. *Brain* **83**, 709–717.

Weiser T & Wienrich M (1996). The effects of copper ions on glutamate receptors in cultured rat cortical neurons. *Brain Res* **742**, 211–218.

White MD, Farmer M, Mirabile I, Brandner S, Collinge J & Mallucci GR (2008). Single treatment with RNAi against prion protein rescues early neuronal dysfunction and prolongs survival in mice with prion disease. *Proc Natl Acad Sci U S A* **105**, 10238–10243.

White MD & Mallucci GR (2009). RNAi for the treatment of prion disease: a window for intervention in neurodegeneration? *CNS Neurol Disord Drug Targets* **8**, 342–352.

Wong BS *et al.* (2003). Mapping the antigenicity of copper-treated cellular prion protein with the scrapie isoform. *Cell Mol Life Sci* **60**, 1224–1234.

Woolf CJ & Thompson SW (1991). The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* **44**, 293–299.

Xiao Z, Brose J, Schimo S, Ackland SM, La Fontaine S & Wedd AG (2011). Unification of the copper(I) binding affinities of the metallo-chaperones Atx1, Atox1, and related proteins: detection probes and affinity standards. *J Biol Chem* **286**, 11047–11055.

Xu J *et al.* (2008). Control of excitatory synaptic transmission by C-terminal Src kinase. *J Biol Chem* **283**, 17503–17514.

Yang CR & Svensson KA (2008). Allosteric modulation of NMDA receptor via elevation of brain glycine and D-serine: the therapeutic potentials for schizophrenia. *Pharmacol Ther* **120**, 317–332.

You H *et al.* (2012). Aβ damages neurons by altering copper-dependent prion protein regulation of NMDA receptors. *Proc Natl Acad Sci U S A* **109**, 1737–1742.

Zhang W, Howe JR & Popescu GK (2008). Distinct gating modes determine the biphasic relaxation of NMDA receptor currents. *Nat Neurosci* **11**, 1373–1375.

Acknowledgements

G.W.Z. and P.K.S. are Alberta Innovates–Health Solutions (AIHS) Scientists and hold Canada Research Chairs. Work from the authors' laboratories discussed in this review was funded by the Canadian Institutes of Health Research, The Alberta Prion Research Institute, and PrioNet Canada. H.Y. is an AIHS post-doctoral fellow.