● PERSPECTIVES

GluN2B-NMDA receptors in Alzheimer's disease: beyond synapse loss and cell death

Alzheimer's disease (AD) is one of the most devastating diseases affecting the life and health of aging population. Two hallmarks of AD are senile plaques and neurofibrillary tangles, and AD is well known for the massive loss of neurons and impaired cognitive functions especially memory loss. Despite extensive search for effective treatment, available drugs have limited efficacy without affecting the course of AD. Significant efforts have been devoted to curb the production of amyloid β (Aβ; the major component of plaques) or enhance the clearance of it, with the aim to reduce the accumulation of plaque in the brain. Antibodies that can bind Aβ to increase their removal have received a lot of attention although recent clinical trial results have been largely negative and disappointing (Panza et al., 2014). Targets that are not directly related to Aβ have also been pursued. One such target is N-methyl-D-aspartate (NMDA) receptors (NMDARs), a subclass of glutamate receptors. The antagonist of NMDAR memantine has been approved for treating moderate to severe AD, although the exact mechanism underlying its action is still in debate (Kotermanski and Johnson, 2009).

Due to its unique properties that its activation requires both binding of glutamate and postsynaptic depolarization, NMDARs have been regarded as the "coincidence detector" and hence play critical roles in synaptic plasticity, memory functions and the refinement of neuronal connections during development (Paoletti et al., 2013). It is well established that excessive activation of NMDARs can lead to neuronal death, generally defined as excitotoxicity. It has been a recent debate whether the NMDARs that mediate this excitotoxicity are unique in some ways, such as their subunit composition (*e.g.*, containing GluN2B) or subcellular locations (*e.g.*, extrasynaptic regions; Zhou and Sheng, 2013). The majority of excitatory synapses on excitatory neurons in the neocortex and hippocampus are located on dendritic spines, and spine loss is highly correlated with the reduction in cognitive function in AD patients. There is strong evidence that GluN2B-NMDARs is involved in neurodegeneration and Aβ-induced synaptic dysfunction and synapse loss in AD, and inhibition of GluN2B-NMDARs appears to prevent or reverse some of the deficits (Paoletti et al., 2013; Zhou and Sheng, 2013). Antagonists to GluN2B-NMDAR have potential therapeutic values to provide neuroprotection and improve cognitive function in AD patients. Tau has been shown to be required for the localization of fyn tyrosine kinase to dendritic spines, where it phosphorylates GluN2B-NMDARs which leads to enhanced association of GluN2B-NMDAR with PSD-95 and downstream neurotoxic effects. Disrupting the interaction between GluN2B and PSD-95 *in*

vivo improved memory functions and reduced premature death in AD mice (Zhou and Sheng, 2013). However, the majority of the supporting evidence has been gathered from cultured neurons or acute brain slices in response to high concentrations of acute exogenous Aβ (usually on a time course of hours). Whether long-term *in vivo* treatment of AD mouse models with GluN2B antagonists is beneficial has not been reported: this is a key test in evaluating the potential therapeutic value of GluN2B antagonists in AD.

To address this directly, we used piperidine18 (Pip18), a potent and selective GluN2B-NMDAR antagonist with favorable pharmacokinetic properties (Hanson et al., 2014). Sharing the same mode of action with the widely used GluN2B antagonists Ro25-6981 and ifenprodil , the blockade of GluN2B-NMDARs is achieved *via* allosteric modulation. Neither short-term (17 days) nor long-term (4 months) treatment with Pip18 in two different AD mouse models resulted in any improvement in cognitive functions (as measured by spatial learning and fear conditioning) or spine loss associated with plaques. It is possible that GluN2B antagonists need to be administered earlier (prior to accumulation of plaque) to affect pathogenesis. To address this, we treated 3-month-old AD mice with Pip18 for 2 months, but did not observe any effect on spine loss associated with plaques. As an indication of bioavailability of Pip18 in the brain, both AD and wild type mice lost body weight, and wild type mice showed increased anxiety-like behavior. Poor efficacy of GluN2B antagonists in AD models challenges the long-held expectation of the therapeutic potential for GluN2B-NMDAR antagonists in AD.

The alterations of neural functions in wild type mice by GluN2B antagonists is worthy of more discussion. In a different study, we found that acute treatment with GluN2B antagonists Ro25-6981 impaired Y-maze performance in wild type mice, and chronic treatment led to impaired *in vitro* gamma oscillations (Hanson et al., 2013). But we did not observe any benefit, either acutely or chronically, in a Down syndrome model (a mental retardation and early-onset AD model) with these treatments. There are two other interesting and important findings in this study: (1) acute effects of GluN2B antagonists are often the opposite of chronic effects, both *in vitro* and *in vivo*; (2) activation of GluN2B-NMDARs on the GABAergic inhibitory interneurons affects the level of inhibition and hence contributes to the balance between excitation and inhibition in the neural circuitry.

There are a few important lessons learned from the above studies: (1) it highlights the importance of using the appropriate models to study disease mechanism and identify therapeutic targets. For example, acute application of high concentration Aβ onto developing neurons *in vitro* does not mimic gradual increase in Aβ concentrations in the mature brain. (2) It also puts certain doubts into the notion that activation of GluN2B-NMDARs is a major cause of excitotoxicity in chronic neurodegenerative diseases (such as AD), although this type of excitotoxicity may have significant contribution to neurodegeneration associated with acute and large elevation in the extracellular glutamate concen-

tration (such as in stroke). A recent study found decreased GluN2B-NMDAR phosphorylation (Tyr1472) and reduced Src activity in young AD mice, suggesting a reduced activity/presence of GluN2B-NMDARs which could explain the lack of benefit of GluN2B antagonists (Mota et al., 2014). (3) It points to the critical importance of GluN2B-NMDARs in the proper functioning of neural circuitry due to their presence on the inhibitory, GABAergic interneuorns. GluN2B antagonists reduce synaptic inputs onto the inhibitory neurons, alter the balance between excitation and inhibition, and in turn affect neural network functions (such as gamma oscillations) (Hanson et al., 2013). Therefore, when evaluating the effects of GluN2B antagonists, it is necessary to go beyond their well-known ability to reduce excitotoxicity, and to consider their effects on the neural circuitry; in other words, we need to change from the excitatory neuron-centric view to include other components of the circuitry when considering the therapeutic values of GluN2B antagonists. In addition, when inhibition is altered (such as by GluN2B antagonists), the acute and long-term effects may not be the same since altered inhibition may drive the reorganization of the circuitry. In that sense, the chronic effects of GluN2B antagonists cannot be readily deduced or extrapolated from their acute effects. (4) Rather than memory functions, NMDARs appear to be more critically involved in mood and fear related functions (Riaza Bermudo-Soriano et al., 2012), consistent with our observations that GluN2B antagonists led to altered open field activity (likely reflecting increased anxiety) and impaired active avoidance learning in wild type mice following chronic treatment (Hanson et al., 2014).

Why memantine is an approved AD drug while GluN2B antagonists are likely not? Is it due to the differences in their subunit selectivity? How memantine works in AD is still in debate, various hypotheses have been put forward, from reducing excessive tonic activation but preserving phasic, physiological activation of NMDARs, to preferential targeting GluN2C/2D-NMDARs (more abundantly present on the inhibitory neurons) (Kotermanski and Johnson, 2009). I would like to discuss a possibility that is often overlooked. When discussing the efficacy of AD treatment in animal models, improving cognitive function almost always comes to mind. There are a range of other pathological alterations associated with AD, including neuropsychiatric issues (such as depression, anxiety, agitation). There is evidence that memantine may be beneficial for controlling/reducing some of these psychiatric issues, such as agitation and aggression (Wilcock et al., 2008), and in doing so improves the quality of life in AD patients and perhaps their cognitive ability as well. Animal studies suggest that NMDAR are involved in the extinction of fear related memory, although the exact contribution of GluN2B-NMDARs still needs to be better defined (Kaplan and Moore, 2011). Thus, GluN2B antagonists could be useful in treating neuronal functions in addition to cognition in AD, but it is unknown whether GluN2B antagonists could have similar or even better efficacy than memantine in this regard. From a scientific point of view, it is important to understand whether subunit-selective inhibitors are more useful or effective as a drug than the pan-NMDAR inhibitors, and whether the targeted processes are indeed mediated by NMDARs in a subunit-specific manner.

It is disappointing that a long-term sought after AD target, GluN2B-NMDARs, may not be viable, at least from the point of improving cognitive function and saving synapse. It highlights the challenge of treating a complex disease with a protracted time course. It has also shown us convincingly that a disease with system-wide changes cannot be comprehensively mimicked using cellular or synaptic models. To be disease-modifying, therapeutic interventions need to affect many aspects of the nervous system functions, and this may be better served by engaging multiple targets, such as with epigenetic modulators.

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References

Hanson J, Weber M, Meilandt W, Wu T, Luu T, Deng L, Shamloo M, Sheng M, Scearce-Levie K, Zhou Q (2013). GluN2B antagonism affects interneurons and leads to immediate and persistent changes in synaptic plasticity, oscillations, and behavior. Neuropsychopharmacology 38:1221-1233.

Hanson JE, Meilandt WJ, Gogineni A, Reynen P, Herrington J, Weimer RM, Scearce-Levie K, Zhou Q (2014) Chronic GluN2B antagonism disrupts behavior in wild-type mice without protecting against synapse loss or memory impairment in Alzheimer's disease mouse models. J Neurosci 34:8277-8288.

Kaplan GB, Moore KA (2011) The use of cognitive enhancers in animal models of fear extinction. Pharmacol Biochem Behav 99:217-228.

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

Mota SI, Ferreira IL, Valero J, Ferreiro E, Carvalho AL, Oliveira CR, Rego AC (2014) Impaired Src signaling and post-synaptic actin polymerization in Alzheimer's disease micehippocampus - Linking NMDA receptors and the reelin pathway. Exp Neurol 261:698-709.

Panza F, Solfrizzi V, Imbimbo BP, Logroscino G (2014) Amyloid-directed monoclonal antibodies for the treatment of Alzheimer's disease: the point of no return? Expert Opin Biol Ther 30:1-12.

Paoletti P, Bellone C and Zhou Q (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and diseases. Nat Rev Neurosci 14:383-400.

Riaza Bermudo-Soriano C, Perez-Rodriguez MM, Vaquero-Lorenzo C, Baca-Garcia E (2012) New perspectives in glutamate and anxiety. Pharmacol Biochem Behav 100:752-774.

Wilcock GK, Ballard CG, Cooper JA and Loft H (2008) Memantine for agitation/aggression and psychosis in moderately severe to severe Alzheimer's disease: a pooled analysis of 3 studies. J Clin Psychiatry 69:341-348.

Zhou Q, Sheng M (2013) NMDA receptors in nervous system diseases. Neuropharmacology 74:69-75.