Mechanosensitivity of N-methyl-D-aspartate receptors (NMDAR) is the key through which amyloid beta oligomers activate them

Giulia Fani, Fabrizio Chiti*

Alzheimer's disease (AD) is one of the major forms of dementia, accounting for 60 to 80% of the cases of dementia, affecting approximately 50 million people worldwide, which is why it has become an object of great interest in both the medical and social fields (Source: Alzheimer's Association). One of the main histopathological hallmarks of AD is the formation and accumulation of senile plaques in the brain parenchyma, mainly composed by fibrillar aggregates of the amyloid β (Aβ) peptide. This peptide is generated by the cleavage of the membrane protein named amyloid β precursor protein, and its secretion in the extracellular space leads to its aggregation into amyloid fibrils. Various intermediates of this process of aggregation have been identified, and it was found that prefibrillar, soluble oligomeric forms of Aβ are more likely to be the pathological species (Benilova et al., 2012).

AD is likely to have a complex pathogenesis involving aberrant neurotransmission systems, such as cholinergic, adrenergic, and glutamatergic network, and their interactions. In this complex scenario, the extrasynaptic N-methyl-D-aspartate receptors (NMDAR) are known to be involved in the neurotoxicity mediated by the Aβ oligomers (Snyder et al., 2005; De Felice et al., 2007; Acosta et al., 2017; Cascella et al., 2017). The NMDAR are ionotropic glutamate receptors that play an important role in excitatory synaptic neurotransmission in the mammalian central nervous system, mediating the vast majority of excitatory transmission in neuronal networks (Scheefhals and MacGillavry, 2018). It is known that neurons affected by Aβ oligomers reduce the density of synaptic NMDAR on the cortical neuronal membrane (Snyder et al., 2005), but in the extrasynaptic regions the aberrant activation of these receptors causes an excessive influx of extracellular Ca^{2+} ions into the cytoplasm, causing the cascade of biochemical changes that lead to the neuronal dysfunction and death observed in the disease (Acosta et al., 2017). This is particularly evident in moderate and severe AD, where a hyperactivation of NMDAR has been observed (Lin et al., 2019).

Activation of extrasynaptic NMDAR was early recognized as one of the most important events in AD and, in fact, one of the drugs approved by the American Food Drug Agency, the European Medicine Agency and other governmental drug agencies, for the treatment of AD is Memantine, a non-competitive NMDAR antagonist. But how does the excessive activation of these receptors occur in AD? This is a topic that has posed a great challenge in the scientific community.

It is well known that one of the dysfunctions occurring in AD, that is given full attention in context of NMDAR activation, is the excitotoxicity process, according to which the extrasynaptic NMDAR are excessively activated by the increased levels of glutamate in the extracellular space, caused by excessive glutamate release from astrocytes and inhibition of its reuptake by the same astrocytes and neurons (Conway, 2020). However, another additional mechanism of NMDAR activation is emerging in AD: that caused directly by Aβ oligomers. In this process, the oligomers do not interact physically with the receptors, nor do other proteins mediate this oligomer-induced NMDAR activation. Rather, the oligomers interact with the lipid bilayer of the cell membrane and the NMDAR "sense" their altered physical properties by their known, yet often neglected, mechanosensitivity (Fani et al., 2021). In the next sections we will describe these processes in more detail.

Glutamate excitotoxicity: Excitatory glutamatergic neurotransmission via NMDAR is critical for synaptic plasticity and neuron survival (Conway, 2020). However, their excessive activation with large elevations of intracellular Ca^{2+} levels, leads to gradual loss of synaptic function and ultimate neuronal cell death. Glutamate uptake and recycling system is an important factor for signaling processes and in AD this system can be severely damaged. Aβ oligomers were found to reduce the glutamate reuptake in astrocytic cells and neurons by the excitatory amino acid transporters 1 and 2, leading to the reduction of glutamate clearance. This originates from the Aβ-induced reduction of the excitatory amino acid transporters 1/2 expression, or their surface localization, with mislocalization and internalization in astrocytes (Conway, 2020). Glutamate uptake is also altered for the pro-inflammatory cytokines potentially released from activated microglial cells in the presence of Aβ oligomers. Moreover, the cystine/glutamate transporter (x_c^-), mediating the release of glutamate by astrocytes, is upregulated, leading to the increase of glutamate release. The combined effects of impaired glutamate uptake and enhanced glutamate release cause an excessive glutamate concentration in perisynaptic space, which activates the extrasynaptic NMDAR leading to a rise in intraneuronal Ca^{2+} concentration (Conway, 2020).

Aβ oligomer-induced membrane destabilization: Experimental evidence has shown, however, that the central role of the extrasynaptic NMDAR in AD is not only for their

excessive activation through excitotoxicity, but also for a direct activation induced by the Aβ oligomers on the neuronal membrane (De Felice et al., 2007; Demuro et al., 2010; Acosta et al., 2017; Cascella et al, 2017; Fani et al., 2021). Many glutamate-independent mechanisms have been described through which Aβ oligomers cause an early influx of Ca^{2+} ions from the extracellular space into the cytosol. It is indeed known that the mechanism of toxicity of the Aβ oligomers include the interaction with the cellular membrane and the consequent destabilization of the lipid and protein components. Evidence on the perforation and destabilization of the cellular membrane, with a direct passage of the Ca^{2+} ions through the membrane, has been reported using different cellular lines or membrane models (Relini et al., 2004; Demuro et al., 2010; Cascella et al., 2017). Aβ oligomers have also been reported to induce increased calcium influx via the activation of different Ca^{2+} channels on the cellular membrane, such as the voltage-dependent Ca^{2+} channels, the transient receptor potential melastatin 2, the transient receptor potential A1, the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, or the NMDAR (see Fani et al. 2021 and references therein). In particular, the involvement of the NMDAR has been repeatedly reported and shown to be one of the major channels responsible for Aβ-induced Ca^{2+} influx, particularly in the early stages of Ca^{2+} influx (De Felice et al., 2007; Fani et al. 2021). Indeed, the time-dependent rise of cytosolic Ca^{2+} ions showed the appearance of a lag phase following the inhibition of the receptor with memantine (Fani et al., 2021).

Oligomers and NMDAR: Provided that NMDAR are activated by Aβ oligomers, the question remains as to the mechanism through which such an activation occurs, which has long been studied. It has been observed with confocal microscopy or co-immunoprecipitation that the oligomers are able to bind to the lipid rafts of the membrane enriched with NMDAR (De Felice et al., 2007; Cascella et al 2017), but a lack of their direct interaction was shown with fluorescence enhancement energy transfer, in which the receptors and the oligomers were labeled with a donor and acceptor probe, respectively (Fani et al., 2021). Moreover, none of the protein subunits forming the NMDAR are present in the oligomer interactome studied in N13 cells, despite the presence of these two receptors in this cell line, confirming the absence of a physical interaction between oligomers and NMDAR (Mannini et al., 2019).

How can then misfolded protein oligomers activate extrasynaptic NMDAR in the absence of any direct interaction? A clue to resolve this puzzle comes from the well-established, but often neglected if not even forgotten, mechanosensitivity of NMDAR (Paoletti and Ascher, 1994; Casado and Ascher, 1998; Kloda et al., 2007; Johnson et al., 2019). Specifically, the NMDAR can be potentiated by well-defined mechanical stimuli applied to the cell membrane, such as membrane depression, hypotonic solutions and lateral membrane stretch, whereas the opposite stimuli inhibit them. The mechanical stimulus transmitted to NMDAR via the lipid bilayer is enough to modulate the channel activity

without interaction with other proteins or even agonists (Paoletti and Ascher, 1994; Casado and Ascher, 1998; Kloda et al., 2007; Johnson et al., 2019). Considering both the membrane destabilization mediated by the oligomers and the mechanosensitivity of NMDAR, we wondered if there was a direct interconnection between these two mechanisms in the oligomer-mediated activation of NMDAR.

Mechanical activation of NMDAR: To verify the hypothesis that oligomers may change the physical properties of the cell membrane as a consequence of their interaction with the bilayer and this change results in NMDAR activation, we added specific compounds to the cell membrane known to cause its stretching or compression, to observe variation in the influx of Ca^{2+} (Fani et al., 2021). In particular, we enriched the cellular membrane with two different lipids: lysophosphatidylcholine, with a "cone" shape characterized by a big polar head, which causes a compression of the phospholipid bilayer inhibiting NMDAR, and arachidonic acid, which has the shape of an "inverted cone" with a small polar head, and has the ability to cause a stretch of the lipid bilayer, with the consequent opening of the receptors without acting as a specific ligand (Casado and Ascher, 1998). We observed the ability of lysophosphatidylcholine to neutralize the oligomer-induced activation of the NMDAR (**Figure 1**), suggesting that the opposing force exerted by the lipids effectively inhibits the mechanical signal generated by the action of the oligomers onto the bilayer, changing the membrane tension energetically transmitted to the receptors (Fani et al., 2021). By contrast, arachidonic acid, which themselves determines the passage of Ca^{2+} through the NMDAR, did not have an additive effect with the oligomers (**Figure 1**), suggesting the same mechanism of action by a membrane stretch induction operates (Fani et al., 2021).

To obtain an independent line of evidence that the oligomers cause a lateral mechanical stretch on the plasma membrane, we added the 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate probe embedded within the polar region of the bilayer (Fani et al., 2021). We observed that the rotational freedom of the probe was significantly increased in the presence of the oligomers, indicating an increase of free space between the various lipids and therefore an increased membrane fluidity, again suggesting that a stretch is occurring after the interaction of the oligomers with the bilayer (Fani et al., 2021).

Summary: All the evidence described above, leads us to conclude that Aβ oligomers activate the glutamatergic receptors following the binding and the consequent insertion of the oligomers in the lipid bilayer, which is an event that causes a change of the mechanical properties of the membrane, increasing the lipid fluidity and inducing a physical stimulus that is reminiscent of a mechanical lateral stretching of the whole bilayer. This is transmitted down to the receptors, which are therefore activated due to their mechanosensitivity in the absence of any direct interaction with the oligomers or other protein mediators. This process deserves deepened investigation in future studies.
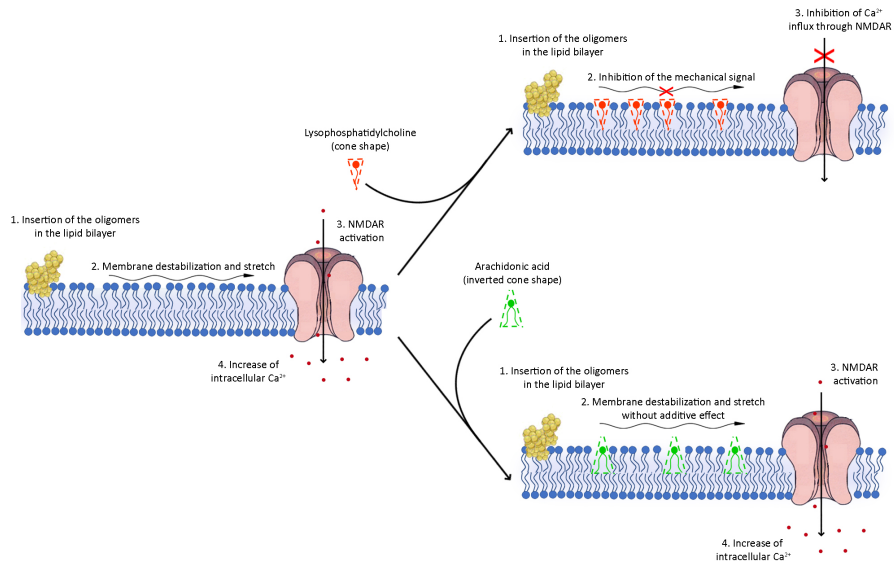
Figure 1 │ **Representative images of the Aβ oligomer-induced activation of NMDAR through insertion of the oligomers in the lipid bilayer and its consequent destabilization, sensed by the NMDAR due to their mechanosensitivity.**
The lysophosphatidylcholine (cone shape) insertion neutralizes the oligomer-induced activation of the NMDAR, due to the opposing force exerted by the lipids which effectively inhibits the mechanical signal generated by the oligomers. By contrast, the arachidonic acid (inverted cone shape) insertion, does not have an additive effect with the oligomers as the oligomers are already "mechanosensitively" activated, suggesting the same mechanism of action. Aβ: Amyloid β; NMDAR: N-methyl-D-aspartate receptors.

Giulia Fani, Fabrizio Chiti[*]
Department of Experimental and Clinical Biomedical Sciences, Section of Biochemistry, University of Florence, Florence, Italy
*Correspondence to: Fabrizio Chiti, D.Phil., fabrizio.chiti@unifi.it.
https://orcid.org/0000-0002-1330-1289 (Fabrizio Chiti);
https://orcid.org/0000-0003-2831-7811 (Giulia Fani)

References

Acosta C, Anderson HD, Anderson CM (2017) Astrocyte dysfunction in Alzheimer disease. J Neurosci Res 95:2430-2447.

Benilova I, Karran E, De Strooper B (2012) The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15:349-357.

Casado M, Ascher P (1998) Opposite modulation of NMDA receptors by lysophospholipids and arachidonic acid: common features with mechanosensitivity. J Physiol 513:317-330.

Cascella R, Evangelisti E, Bigi A, Becatti M, Fiorillo C, Stefani M, Chiti C, Cecchi C (2017) Soluble oligomers require a ganglioside to trigger neuronal calcium overload. J Alzheimers Dis 60:923-938.

Conway ME (2020) Alzheimer's disease: targeting the glutamatergic system. Biogerontology 21:257-274.

De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, Klein WL (2007) Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. J Biol Chem 282:11590-11601.

Demuro A, Parker I, and Stutzmann GE (2010) Calcium signaling and amyloid toxicity in Alzheimer disease. J Biol Chem 285:12463-12468.

Fani G, Mannini B, Vecchi G, Cascella R, Cecchi C, Dobson CM, Vendruscolo M, Chiti F (2021) Aβ oligomers dysregulate calcium homeostasis by mechanosensitive activation of AMPA and NMDA receptors. ACS Chem Neurosci 12:766-781.

Johnson LR, Battle AR, Martinac B (2019) Remembering mechanosensitivity of NMDA receptors. Front Cell Neurosci 13:533.

Kloda A, Lua L, Hall R, Adams DJ, Martinac B (2007) Liposome reconstitution and modulation of recombinant N-methyl-D-aspartate receptor channels by membrane stretch. Proc Natl Acad Sci U S A 104:1540-1545.

Lin CH, Chiu CC, Huang CH, Yang HT, Lane HY (2019) pLG72 levels increase in early phase of Alzheimer's disease but decrease in late phase. Sci Rep 9:13221.

Mannini B, Vecchi G, Labrador-Garrido A, Fabre B, Fani G, Franco JM, Lilley K, Pozo D, Vendruscolo M, Chiti F, Dobson CM, Roodveldt C (2019) Differential interactome and innate immune response activation of two structurally distinct misfolded protein oligomers. ACS Chem Neurosci 10:3464-3478.

Paoletti P, Ascher P (1994). Mechanosensitivity of NMDA receptors in cultured mouse central neurons. Neuron 13:645-655.

Relini A, Torrassa S, Rolandi R, Gliozzi A, Rosano C, Canale C, Bolognesi M, Plakoutsi G, Bucciantini M, Chiti F, Stefani M (2004) Monitoring the process of HypF fibrillization and liposome permeabilization by protofibrils. J Mol Biol 338:943-957.

Scheefhals N, MacGillavry HD (2018) Functional organization of postsynaptic glutamate receptors. Mol Cell Neurosci 91:82-94.

Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 8:1051-1058.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y