Journal Club

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Role of Neurexins in Alzheimer's Disease

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Review of Cvetkovska et al.

Alzheimer's disease (AD) is a prevalent neurodegenerative disease that predominantly affects the elderly worldwide. The general average life expectancy has been increasing in the last decades, and with it the prevalence of age-related diseases such as AD, making it imperative to expand our knowledge on the mechanisms, many of which are still unknown.

Two main proteins create aggregates in AD pathology: tau and amyloid-β (Aβ). The latter is hypothesized to trigger AD pathogenesis. Aβ is a cleavage product of amyloid precursor protein (APP). The physiological role of this protein has recently begun to be uncovered, and it has been linked to synapse formation, among other processes (Müller et al., 2017). More than 25 mutations in APP, as well as gene duplications and chromosome 21 trisomy, have been identified as causative of the familial form of AD. The amyloid hypothesis postulates that Aβ, in various forms, triggers a cascade harming synapses and ultimately neurons, leading to AD pathology. This hypothesis remains the most prevalent in the field, with a recent shift toward defining soluble Aβ oligomers as the toxic agent, rather than plaques (Ricciarelli and Fedele, 2017; Tcw and Goate, 2017).

APP has two proteolytic pathways, mediated by α- and β-secretases, which result in the production of soluble forms of APP, sAPPα and sAPPβ, as well as Aβ, p3, and APP intracellular domain/C-terminal fragment γ. The pathway that leads to the cleavage of Aβ is mediated by the β-secretase and is referred to as the amyloidogenic pathway (Barage and Sonawane, 2015). Aβ pathology occurs at synapses, which have been described as early sites of accumulation of the Aβ42 isoform and is linked to tau pathogenesis. This has led to the hypothesis that the extracellular accumulation of Aβ42 can upregulate the intracellular production of Aβ42 in nearby synapses, thus propagating the disease (Takahashi et al., 2010). In line with this hypothesis, Aβ has been found to bind to synapses, where it accumulates and mediates dysfunction by directly impairing the clearing of both the aggregates and the dystrophic synapses (Sanchez-Mico et al., 2021).

Previous studies have shown that Aβ oligomers can interact with neurexins and disrupt their function, suggesting a link between neurexins and AD pathology (Lee et al., 2020). Neurexins are a family of cell adhesion proteins, present in the presynaptic terminal of neurons. These proteins have been shown to have a role in synaptogenesis by interacting with postsynaptic ligands, such as neuroligin or leucine-rich-repeat transmembrane neuronal proteins (LRRTMs; Gomez et al., 2021). The interactions with these, through competition and coordination at the synapse, regulate the distribution of glutamate receptors (Nozawa et al., 2022). The genes for neurexins are some of the largest in mammals and contain multiple conserved sites of alternative splicing. The main products encoded in each gene are Neurexinα (Nrxα) and Neurexinβ (Nrxβ; Reissner et al., 2013). Neurexins have been found to bind to Aβ oligomers through the following two domains: histidine-rich domain of Nrxβ1/2/3 and inserts at splicing site 4 of Nrx1/2 (Naito et al., 2017). The same study showed that Aβ overexpression in transgenic mice leads to a decrease in the presynaptic expression of Nrxβ, which may be related to the synaptic dysfunction seen in AD pathology. Another study suggests that by blocking the Aβ–neurexin interactions it is possible to prevent Aβ-induced memory impairment in mice (Brito-Moreira et al., 2017). Moreover, both neurexins and neurologins have been recently used as biomarkers for early diagnostics in neurodegenerative diseases, including AD (Camporesi et al., 2022).

Given these previous results, Cvetkovska et al. (2022) focused their research on the interactions between APP and neurexin with regard to the physiological function of presynaptic differentiation of APP, a mechanism previously unexplored. They started by assessing the affinity of APP for neurexins. After showing that soluble APPα and APPβ both show a higher affinity for Nrxβ (comparable to canonical Nrxβ ligands) than Aβ oligomers, Cvetkovska et al. (2022) tested whether the affinity was such that it competed against normal ligands. When affinity to Nrxα was tested, the results were not significant. They then

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compared APP affinity to Nrxβ to that of neuroligin-1 (NL1), the canonical ligand of Nrxβ1, and found that the proteins compete, potentially through a common binding site or an overlapping site. Furthermore, as it is known that NL1 binds to Nrxβ1 in a calcium-dependent fashion at the LNS (Laminin Neurexin Sex hormone-binding protein domain), they next examined to see whether the same is true for APP binding. Indeed, APP was found to bind to the same site in a similar calcium-dependent and splicing-dependent manner to NL1, showing it to be a specific ligand for Nrxβ. This high-affinity Nrxβ binding site for APP was also found to differ from the low-affinity binding site of Aβ oligomers.

The interaction between neurexins and their ligands is mainly mediated by neurexin protein domains (Bourne and Marchot, 2014). Interestingly, a study has shown that modifications on heparan sulfate (HS), a proteoglycan present on neurexins, can also play a role in these interactions. This study has shown that HS modifications are a binding site for neuroligin and LRRTMs and are required for the formation of synapse-organizing complexes. Indeed, mice lacking HS modification in neurexins show deficits at central synapses (Zhang et al., 2018). By either allowing or blocking these modifications, Cvetkovska et al. (2022) showed that APP also binds to HS modifications on neurexin. In line with the study mentioned before, the authors also show that APP binding to HS modifications is a requirement for transcellular axonal recruitment (i.e., the ability of axons to form synapses with other neurons), as the neurons that had HS modifications blocked were not recruited. The inhibition of transcellular axonal recruitment leads to lower adaptability of the CNS, and thus might lead to an impairment of complex behaviors and cognitive processes. Additionally, blocking the binding site on APP stopped the recruitment of Nrxβ1. Both findings show that synaptogenesis mediated by neurexin–APP interaction is dependent on both binding sites. Accordingly, knockdown of neurexin reduced the ability of APP to trigger synaptogenic activity.

Cvetkovska et al. (2022) continued their research by confirming previous findings that APP can induce presynaptic differentiation of both glutamatergic and GABAergic axons. This, along with the presence of Nrxβ in both types of axons, leads them to conclude that this differentiation process may be mediated by the complex formed by APP and Nrxβ. These were convincing *in vitro* results; however, arriving at this conclusion solely on the basis of the concomitant presence of APP and neurexins in the areas involved does not seem conclusive enough to be considered a causal relationship. *In vivo* validation, as well as more in-depth research into other possible factors should be the next steps in this direction.

As discussed earlier, Aβ has been previously shown to interact with neurexins. In the study reviewed here, APP has also been shown to interact with neurexins in a physiological way to mediate synaptogenesis. In AD, the altered presence of Aβ could disrupt this interaction with full-length APP, contributing to AD pathology. To better understand these interactions and to be able to take advantage of this knowledge, further research is necessary.

Since AD is not the only disease that has been linked to neurexins, it is of significance to study its potential therapeutic applications. The spectrum of phenotypes associated with heterozygous deletions of Nrx1 (NRXN1) is diverse and includes the following: (1) autism spectrum disorder (Dachtler et al., 2014); (2) intellectual disability (Zweier et al., 2009); (3) seizures (Fang et al., 2016); and (4) schizophrenia (Owczarek et al., 2015). Additionally, as Cvetkovska et al. (2022) pointed out, the trisomy of chromosome 21 that Down Syndrome patients have makes them especially vulnerable to APP pathology, including AD. Since it is now known that neurexins interact with APP, our team believes Down Syndrome models to be particularly good candidates for any *in vivo* future experiments, an approach that is yet to be explored.

Another promising angle for neurexin research is the ontological development of neurexin functions. It is known that many proteins will have different functions or affinities to ligands during development than the ones they have during adulthood. Considering the emergent studies on neurexin–APP interaction and its role in synaptogenesis, it is conceivable that the mechanisms of interaction change during development, giving us more insight into how those mechanisms might be impaired in neurodegenerative diseases such as AD. Learning more about whether this is the case for neurexin proteins will open the door for identifying new potential ligands that could become important targets during pathologic situations.

To conclude, Cvetkovska et al. (2022) have found that the synaptogenic activity of APP is mediated by Nrxβ. The altered levels of APP present during AD pathology, as well as its cleavage by-products, might disrupt this mechanism, thus contributing to the decay of synapses and subsequent neurodegeneration that characterizes this disease. By learning more about these interactions, it might be possible to find new therapeutic targets and novel research pathways regarding the physiological role of neurexin–APP interactions in AD.

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