Alzheimer's disease: An update of the roles of receptors, astrocytes and primary cilia (Review)

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Abstract. The pathophysiological mechanisms underlying the onset and inexorable progression of the late-onset form of Alzheimer's disease (AD) are still the object of controversy. This review takes stock of some most recent advancements of this field concerning the complex roles played by the amyloid-β (Aβ)-binding p75 neurotrophin receptor ($p75^{NTR}$) and calcium-sensing receptor (CaSR) and by the primary cilia in AD. Apart from their physiological roles, $p75^{NTR}$ is more intensely expressed in the hippocampus of human AD brains and Aβ-bound $p75^{NTR}$ triggers cell death, whereas Aβ-bound CaSR signalling induces the *de novo* synthesis and release of nitric oxide (NO), vascular endothelial growth factor (VEGF)-A and Aβ peptides (Aβs), particularly on the part of normal adult human astrocytes. The latter effect could significantly increase the pool of Aβ- and NO-producing nerve cells favouring the progressive spread of a self-sustaining and self-reinforcing 'infectious' mechanism of neural and vascular (i.e. blood-brain barrier) cell damage. Interestingly, primary cilia concentrate $p75^{NTR}$ receptors in their membranes and are abnormally structured/damaged in transgenic (*Tg*) AD-model mice, which could impact on the adult neurogenesis occurring in the dentate *gyrus*'s subgranular zone (SGZ) that is necessary for new memory encoding, thereby favouring typical AD cognitive decline. Altogether, these findings may pave the way to novel therapeutic approaches to AD, particularly in its mild cognitive impairment (MCI) and pre-MCI stages of development.

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1. Introduction

Almost 2% of the people of Western industrialized countries are affected by Alzheimer's disease (AD) (1,2). But what is this ailment that threatens a growing number people in our aging populations? It is a very slowly expanding neurodegenerative process that betrays its presence by disconnecting and ultimately destroying neural networks in the hippocampus, the brain's ancient memory-recording and accessing 'machine' (3,4). The by far most common late onset AD (LOAD) cases account for over 70% of dementia cases in individuals >70 years of age (5). The incidence of AD increases exponentially with age and doubles every 5 years after the age of 65 (1). In the rare early-onset familiar AD (EOFAD) cases genetic mutations support an Aβ peptide overproduction (1,2). The LOAD pathogenesis is still controversial; it begins 30-40 years before the phenotypic emergence of clinical symptoms, in the entorhinal cortex and the dentate *gyrus*, where the aggregation-prone $Aβ_{1-42}$ peptides (Aβs), which derive from the sequential activity of two proteases, BACE1 and γ-secretase, on the amyloid precursor protein (APP), progressively disrupt the neuronal networks (3-8).

In normal brains, neurons release at synapses nontoxic $Aβ_{42}$ monomers, the physiological levels of which are kept at low, safe levels by various clearance mechanisms involving the activation of proteases, phagocytosis by microglia and dumping into the blood by transporters such as LRP1 (4). But in the aging brains of susceptible persons the $Aβ_{42}$ clearing mechanisms start to fail and the accumulating $Aβ_{42}$ monomers will aggregate into toxic soluble oligomers and protofibrils driving the brain into the onset of the pathology (3,4). Thus, AD starts stealthily maybe as early as during childhood in a subcortical region such as the *locus coeruleus*, from which a prion-like tau mutant would progressively spread and/or maybe

in the brain's default mode network (DMN), which includes the medial temporal memory-recording region (3,4-10). Aβs overproduction associates with the dangerous spread of phosphorylated tau protein (3,4,6). Ultimately, the functionally disturbed neurons cause a lethal accrual of the toxic prion-like pE $Aβ_{3-34}$ (pyroglutamyl $Aβ_{3-34}$) along with the Aβs oligomers with which it associates (11-15). Indeed pE $Aβ_{3-34}$ is likely 'the AD's hatchet man' as it has been called by Jawhar *et al* (12).

In the present study we aim at updating some of the mechanisms supporting AD development and progression, i.e. i*)* the interactions of two nerve cell membrane receptors with Aβs and their effects; ii) the complex involvement of a perhaps unduly overlooked class of glial cells, the astrocytes, in AD; and iii) the involvement of the primary cilia of neurons and astrocytes alike in AD. Here we shall briefly review such topics in their own contexts.

2. Nerve cell membrane receptors in AD

p75 neurotrophin receptor (p75^{NTR}). The $p75^{NTR}$ is a TNF-family low-affinity receptor for neurotrophins such as nerve growth factor (NGF), neurotrophin (NT)-3, NT-4 and brain-derived neurotrophic factor (BDNF). The interest in $p75^{NTR}$ role, if any, in AD development and progression was triggered by the studies of Yaar *et al* (16,17) and Kuner *et al* (18), who showed that Aβs could bind to both $p75^{NTR}$ monomers and trimers, thereby activating its intracellular signalling and inducing apoptosis in human neuroblastoma cells. At about such early times, we employed neuroblastoma cell clones that did not express any of the neurotrophin receptors or had been engineered to express full-length or various truncated forms of the $p75^{NTR}$ to demonstrate that $p75^{NTR}$ binds Aβs via its extracellular domain and, as a consequence, via its death domain directly signals cell death. In fact, this signaling led to caspase-8 and caspase-3 activation and to reactive oxygen species (ROS) production and cellular oxidative stress (19,20). Moreover, the direct and indirect (inflammatory) mechanisms of neuronal damage by Aβs could interact synergistically, since cytokines released from an activated microglia, like TNF-α and IL-1β, remarkably potentiated the neurotoxic actions of the Aβs/$p75^{NTR}$ signaling (19,20). Altogether, these findings indicated that the privileged targets of the cytotoxic actions of Aβs in AD might be $p75^{NTR}$-expressing neurons endowed with receptors for proinflammatory cytokines (19,20).

Concurrently, by means of the same human neuroblastoma cell clones either devoid of all the neurotrophin receptors or expressing the full-length or variously truncated forms of $p75^{NTR}$, we could prove that the neuronal death induced by the prion protein fragment $PrP_{106-126}$ is mediated through its binding to the extracellular domain of $p75^{NTR}$ and the subsequent signaling of its death domain causing the downstream activation of caspase-8 and production of ROS (20,21). Since then other laboratories have corroborated the idea that the Aβs/$p75^{NTR}$ binding engenders a signaling causing neuronal apoptosis (22,23).

More recently, we demonstrated that, besides binding and activating $p75^{NTR}$ receptors, $Aβ_{1-42}$ and its surrogate active peptide $Aβ_{25-35}$, but not the reverse sequence $Aβ_{42-1}$ peptide, at least double the membrane complement of $p75^{NTR}$ receptors in SH-SY5Y human neuroblastoma cells (24). We concurrently established that $p75^{NTR}$ is overexpressed above the level of corresponding wild-type mice in the hippocampal membranes of two strains of AD transgenic mice, i) in 12-15-month-old AD-triple transgenic (*Tg*) mice (*3*x*Tg*-AD) harboring PS1 (M146V), AβPP (Swe) and tau (P301L) and ii) in 7-month-old B6.Cg *Tg*-AD mice harboring PSEN1dE9 and AβPP (Swe). Importantly, this increase correlated with the age-dependent rise in $Aβ_{1-42}$ levels in the AD mice (24). Evidence was also gained that the $Aβ_{42}$ oligomers known as Aβ-derived diffusible ligands (ADDLs) induced the expression of $p75^{NTR}$ protein via the phosphorylation of insulin-like growth factor-1 receptor (IGF-1R) in SH-SY5Y human neuroblastoma cells (25). An *in vivo* microinjection of ADDLs also increased the $p75^{NTR}$ protein expression by 1.4-fold in the ipsilateral hippocampus as compared to the non-injected contralateral hippocampus. Moreover, in the ADDLs-microinjected mouse hippocampi IGF-1R phosphorylation surged within 30 min, while the co-administration of picropodophyllin, an IGF-1R kinase inhibitor, prevented any ADDLs-induced $p75^{NTR}$ expression from occurring (25). In addition, in the hippocampi of 6-month-old AβPPswe/PS1dE9 *Tg*-AD model mice that had accumulated significant amounts of $Aβ_{1-42}$ a higher $p75^{NTR}$ protein expression together with higher levels of IGF-1R phosphorylation were detected with respect to the hippocampi from age-matched wild-type mice (25). Hence, $Aβ_{42}$ oligomer-mediated IGF-1R activation may trigger an increase in $p75^{NTR}$ protein expression in the hippocampus of a *Tg*-AD mouse model brain during the early stages of disease development.

Notably, these findings raised an important question, i.e. whether the $Aβ_{42}$'s accumulation is also coupled with an increased hippocampal membrane-associated $p75^{NTR}$ expression in human AD brains. Indeed, the mechanisms controlling the hippocampal expression of $p75^{NTR}$ are poorly known. It is a commonly held view that the $p75^{NTR}$ proteins are not expressed by the hippocampal nerve cells, but are carried to the hippocampus via the afferent axons of basal forebrain cholinergic neurons (BFCNs). Yet, BFCNs are selectively killed in the early phases of AD, which would entail a $p75^{NTR}$ fall in the hippocampi of AD brains (26). On the other hand, a high concentration of $p75^{NTR}$ receptors is detectable in the membranes of the primary cilia of dentate *gyrus* granule cells in the mouse hippocampus (27). Others have reported $p75^{NTR}$ protein expression in normal mice granule cell precursors up to the early postmitotic maturation of neuroblasts (28) and in dendritic spines and afferent terminals of hippocampal CA1 pyramidal neurons of normal C57BL/6 mice (29). To solve this question, we used polyclonal and monoclonal antibodies against the $p75^{NTR}$ receptor's intra- and extracellular domains. Thus, we were able to show that the mean level of membrane-associated $p75^{NTR}$ in the hippocampal formation is significantly higher (~2-fold, $p<0.03$) in human AD brains than in identical samples of hippocampal formation in age-matched non-AD human brains (30). As yet, we do not know whether the same types of nerve cells express $p75^{NTR}$ receptors in murine and human hippocampi, respectively. Nevertheless, an elevated membrane-bound $p75^{NTR}$ in the human hippocampus could be another characteristic of AD. It remains to be determined whether and/or how such an increased expression of membrane-bound $p75^{NTR}$ might be

a cause of the hippocampal destruction causing the cognitive decay in AD patients.

Calcium-Sensing Receptor (CaSR). The highly conserved CaSR gene encodes the CaSR protein, which belongs to family C of G-protein-coupled receptors (GPCRs), whose members have no sequence homology with those of other GPCR families (31). The CaSR's huge (>600 amino acids) bilobed extracellular N-terminus domain looks like a Venus Flytrap (VFT), whose lobes are joined via a three-strand hinge to 7 transmembrane α-helices (TM1-TM7) ending with the intracellular C-terminus (32). A cysteine-rich region (CRR) links the VFT to the 7TM region and is important for signal transmission from the VFT-like domain to the TM1-TM7 (33). CaSR's intracellular tail includes two regions essential for its cell surface expression and biological activity (34). By rearranging the two 7TM regions, ligand binding permits the intracellular CaSRs C-tails to bind various G proteins ($G_{q\alpha}$, $G_{i\alpha}$ and $G_{11\alpha}$) (35). CaSRs form homodimers (CaSR/CaSR) or heterodimers (CaSR/mGluR) in their membrane-bound form, although they can function even as monomers (32). Dimers are assembled at the ER to allow CaSR transport to the plasmalemma (36). The huge VFT lobes of CaSR homodimers cooperatively bind ligands, e.g. Ca^{2+} (35). The CaSR detects changes in $[Ca^{2+}]_e$ (35), but is a non-selective receptor (37). Ca^{2+}, di- and tri-valent cations, antibiotics and polyamines are the CasR-activating orthosteric agonists, whereas endogenous ligands or factors, like pH, ionic strength, $[Na^+]_e$ and aromatic L-α-amino acids are the allosteric CaSR modulators (37). Intracellular signaling, mediated via Ca^{2+} influx, has been connected to MAPK (MEK/ERK and JNK) activation, ion channel function, gene expression, cell proliferation and cell death (35). Most significantly, even Aβs do bind and activate the CaSR (38). Hence, CaSR-expressing neurons and glial cells of all types are susceptible to the cytotoxic effects of the CasR-activating $Aβ_{42}$ oligomers and fibrillar aggregates (39). The interest in CaSR's role in AD pathogenesis has been increasing since the first evidence was gained of $Aβ_{42}$/CaSR interactions in hippocampal pyramidal neurons (40). But CaSR expression by the astrocytes entails deep neuropathologic implications since they play significant roles in inflammatory and degenerative brain diseases (39-41). Using cultured phenotypically stable normal adult human astrocytes freshly isolated from the temporal lobe cerebral cortex we could show that exogenous Aβ-stimulated CaSR signaling triggers i) the expression of nitric oxide synthase-2 (NOS-2); ii) the expression and activity of GTP cyclohydrolase 1 (GCH1), which produces tetrahydrobiopterin (BH4); and iii) the synthesis and release of large amounts of NO on the part of the BH4-dimerized/activated NOS-2 (41-44). In its turn, the overproduced NO can be fairly damaging to neurons and glial cells (see also below).

Moreover, using the same cultured phenotypically locked-in normal adult human astrocytes exposed to normoxic conditions we could demonstrate that the Aβs/CaSR interaction also induces within 18-24 hours the nuclear translocation of the hypoxia-inducible HIF1α/HIF1β transcription complex that drives the expression of three VEGF-A splice variants ($VEGF\text{-}A_{121}$, $VEGF\text{-}A_{165}$ and $VEGF\text{-}A_{189}$) and the increased synthesis and secretion especially of $VEGF\text{-}A_{165}$ (45 and unpublished results).

Finally, and perhaps most interesting of all, the Aβ-activated CaSR signalling also stimulates the normal human adult astrocytes in culture to make significant amounts of their own $Aβ_{42}$ oligomers (46), which accumulate inside the cells but are also released into the medium (our unpublished results). Thus the Aβ/CaSR-evoked signalling can simultaneously modulate the expression/production of NO, VEGF-A and $Aβ_{42}$ in human astrocytes.

3. Astrocytes in AD

Neurons have attracted the most attention from people trying to understand AD pathophysiologic mechanisms (3,4). Obviously they are very important in the AD story and with them die the person's cognition and ultimately other functions. Concerning glial cells, undeniably there are more astrocytes than neurons in the human brain, although there are arguments about the size of this majority that ranges from 1.4- to 10-fold the actual neurons' numbers (47). Until recently, astrocytes have been relegated to simple janitorial roles: they have not been believed to be able to make Aβs, but only to sweep them up and then die if and when they accumulate too much of Aβs (48), as Aβs are supposedly only made and released by the neurons. There is an increasing realization that astrocytes are much more important than previously thought; they actually protect neurons and are in fact the neuron partners in synaptic formation and function. They physically cradle or embrace neurons (Fig. 1) (49), shielding them from the signaling noise of neighboring neurons. They keep their neuron synapses optimally functional by regulating synaptic K^+, by sweeping up secreted neurotransmitters (e.g., glutamate) from the synaptic space and removing transmitters spilled into the nearby space by neighbouring neurons. Astrocytes also collaborate in neuronal signaling with their own gliotransmitters, and they can stimulate synapse formation (47-50).

Recent findings have added a novel facet to this picture (Fig. 2). Astrocytes are no longer just by-standing synapse blankets that only clean up the Aβs released from shattered neurons in, for example, the increasingly cognitively disabled AD brains. Astrocytes are actually stimulated by their neuron Aβs to make and secrete their own Aβs (46 and unpublished results). This means that as astrocyte-contacting neurons in key regions of the brain enter the covert early stages of AD and start over-secreting Aβs, they directly spray the astrocytes with their Aβs. The exciting finding is that this causes the same astrocytes to make and release their own Aβs and spread them to other neurons in local networks, stimulating such neurons to join and enlarge the pool of cells making Aβs. In this way, Aβs-exposed astrocytes act as vectors of a contagious, self-sustaining and Aβs-spreading disease. But this might not last, as the accumulating Aβs released from both neurons and astrocytes reach a level that stimulates the latter cells to start making large amounts of nitric oxide (NO), from which highly toxic peroxynitrites ($ONOO^-$) can be engendered (41-44). Both these diffusible agents damage neurons and astrocytes to the point of inducing cell death. Obviously, the progressive loss of astrocytes besides neurons

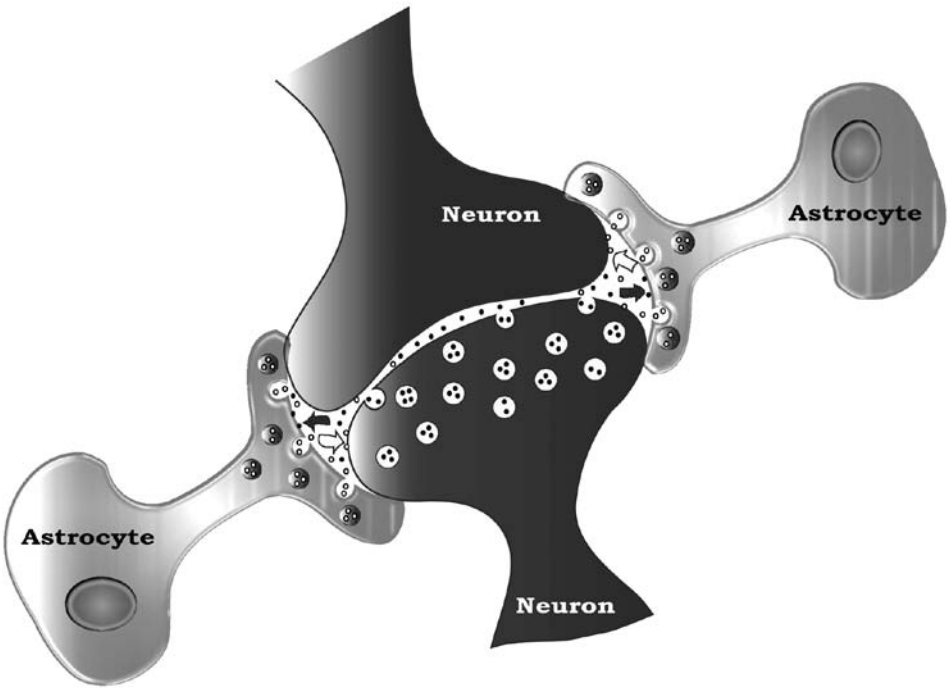

Figure 1. The cradling astrocytes of the tripartite synapse. Each astrocyte, which is gap-junctionally interconnected into a network of astrocytes, cradles ~4-8 neuronal somata and ~$3x10^5$ synapses to optimally configure themselves in response to neuronal activity, insulate their neurons from the signaling noise such as glutamate spilled over from the neighbors, regulate synaptogenesis, clear away and recycle released glutamate and K^+ from the synaptic space and to directly exchange various gliotransmitters (ATP, glutamate, D-serine, TNFα) and most importantly in the present context Aβs (47,49).

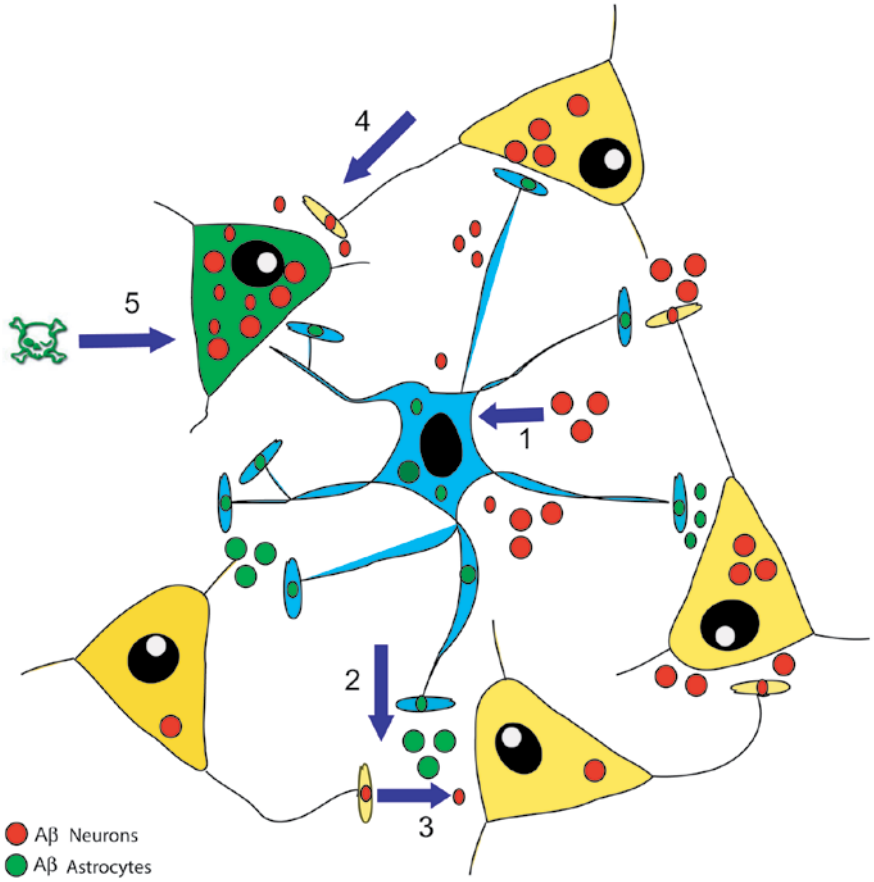

Figure 2. It is known that exogenous $A\beta_{1-42}$ can stimulate both astrocytes and neurons to produce and secrete endogenous $A\beta_{1-42}$ (46 and our unpublished results). Thus it seems likely that because of their intimate association the initiation of $A\beta_{1-42}$ overproduction and synaptic release by a neuron (Step 1) can recruit the production of Aβs by the associated astrocytes (Step 2) which in turn can stimulate other neurons to start making and releasing Aβs (Step 3), inducing the disconnection of so called 'undead' neurons that eventually die (Step 4).

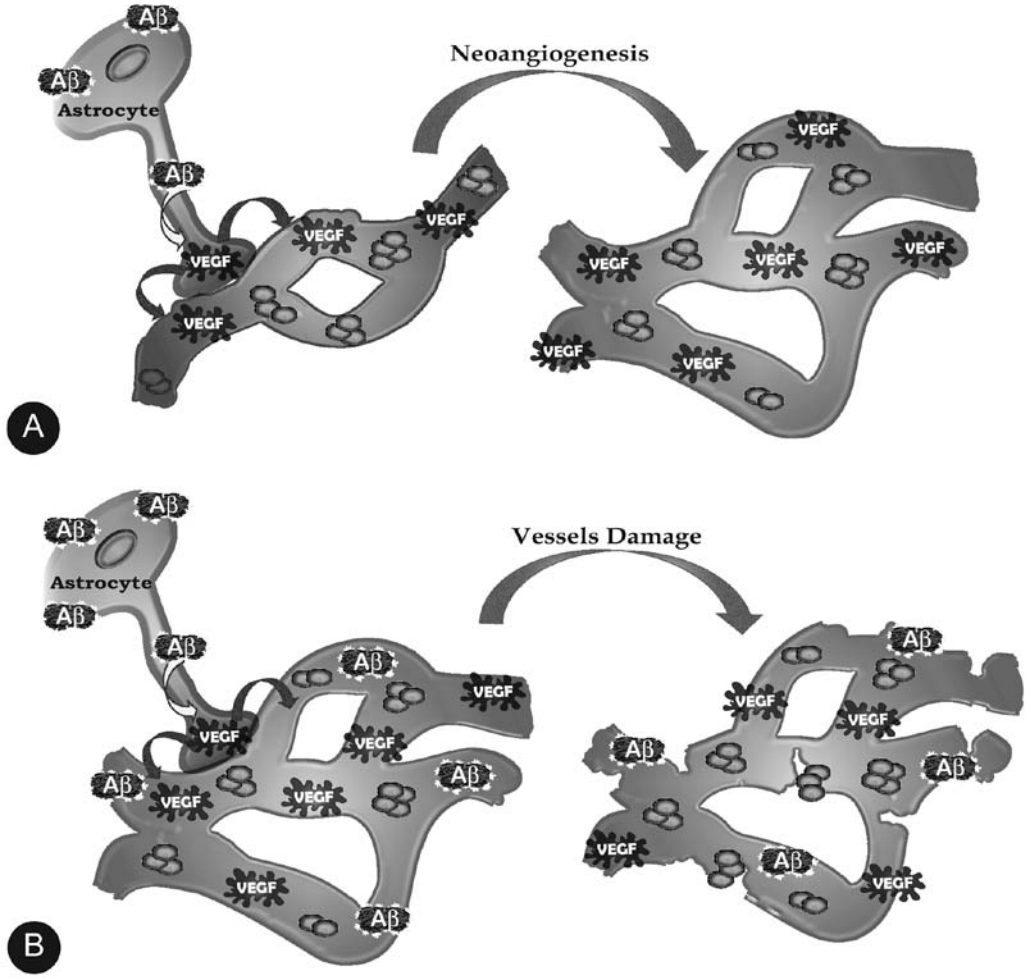

Figure 3. The Aβs accumulating in the preAD (i.e., MCI) brain's hippocampal dentate *gyrus*/CA3 subregions stimulates astrocytes to make and release large amounts of the potently angiogenic VEGF (45). (A) This increases the local vascular density. Stimulating the neuronal activity in this region will increase the blood flow through the expanded vascular network and with it the blood oxygen-dependent (BOLD) fMRI signaling. However, this Aβ-induced angiogenesis will falsely indicate hyperactivity from the hippocampal subregions, which have been caused to atrophy by the accumulating Aβs. (B) However, as the brain converts from MCI to AD, the excessive accumulation of Aβs and the resulting production of large amounts of NO will perforate and sever the blood vessels and break through the blood-brain barrier (60,61).

impairs synaptic function and the provision of supplies from the circulation (47,49,51).

Amongst other activities, astrocytes send information about the activities of the neurons they cradle to their end-feet on local blood vessels, which adjusts the blood flow to provide the glucose and oxygen needed to feed the busy neurons. However, as *in vitro* (45), the accumulating Aβs in the key regions of the pre-AD brain such as the dentate *gyrus* and the CA3 subregions also stimulate astrocytes to make VEGF-A (52-54), which as expected stimulates the growth of blood vessels (Fig. 3). This increase in the local vascular density magnifies the blood flow and the blood oxygen-dependent (BOLD) functional magnetic resonance imaging (fMRI) signaling from an active region such as a hippocampus trying to respond to a dentate *gyrus*/CA3-directed task (55,56). This has been wrongly interpreted as if the already declining hippocampi of AD-bound people with pre-AD mild cognitive impairment (MCI) are hyperactive, which they are not (57-59). Again, in the early stages the VEGF-expanded vascular networks are intact and the blood vessels are functional, but this ends when the accumulating Aβs stimulate the astrocytes (and microglia) to make huge, damaging amounts of NO, which contributes to the perforation and severing of the blood vessels and with this the breaching of the blood-brain barrier and its disastrous consequences for brain function (60,61) (Fig. 3).

4. Nerve cell primary cilia in AD

Contrary to the old neurological tenet, in the brain of adult humans and rodents there are two principal areas in which neurogenesis does occur, the subgranular zone (SGZ) of the dentate *gyrus* and the subventricular zone (SVZ) (62-64). In the hippocampal SGZ a pool of neural stem cells, the astrocyte-like type 1 radial glial cells, are able to produce new granule cells when they are needed for memory encoding (65,66). These cells express some properties of the astrocytes, including glial fibrillary acidic protein (GFAP), the typical astrocytic marker, are endowed with vascular end-feet and occupy their special SGZ niche: the upshot is a blood vessel-associated gap-junctionally interconnected

astrocytic syncytium. Most of these cells possess non-motile, 4-8 μm-long sensory antennas protruding from their bodies, the primary cilia, wrapped in a plasma membrane that is stuffed with various kinds of receptors. Among the receptors found in the rodent granule cell cilia are the $p75^{NTR}$ receptor (10,24,39), the somatostatin type 3 receptor (SSTR3) (67,68) and the Sonic hedgehog (Shh) system's smoothened (SMO) and Patched (Ptch) proteins (65,69-72). Conversely, the neurotrophin tyrosine kinase receptor TrkA does not co-localize in the primary cilia membrane. Signals from the receptors on these cilia are believed to drive several fundamental activities such as neurogenesis, neuroblast maturation and memory encoding. Neurotrophin (e.g., BDNF)-induced $p75^{NTR}$ signalling from the primary cilia drives the proliferation of granule cells precursors in the SVZ of the dentate *gyrus*, as preventing this signalling severely reduces neurogenesis (65,66,73).

How these primary cilia might be involved in AD-linked cognitive deterioration? It appears that the accumulating toxic $A\beta_{42}$ oligomers in AD brains at first stimulate the proliferation of GC progenitors. But later, when such oligomers are caught in the fibrillary plaques, the newly formed neuroblasts cannot mature or ultimately survive (74-76). The failure of the newly generated neurons to mature and the resulting granule cell layer shrinkage and memory failure is likely, at least partly, to be due to the characteristic decline of somatostatin in AD and with it of the cilial SSTR3 signalling needed for memory encoding (65,66). These notions enticed us to surmise that primary cilium damage may cause the crippling decline of new memory formation in AD (77). This view is supported by the striking shortening of dentate *gyrus* granule cell primary cilia linked to the strongly reduced neurogenesis in AD *Tg* mice accumulating both $A\beta_{42}$ and tau protein (78,79).

Moreover, cilial $p75^{NTR}$ can be bound and activated by $A\beta_{42}$ (19,22). This would elicit an initially increased neural progenitor cell proliferation in the early stages of AD (80-82), meanwhile, the hippocampal supply of acetylcholine (Ach) is progressively reduced by the accumulating $A\beta_{42}$ that kills Ach-producing basal forebrain cholinergic septal neurons (BFCSNs) (83). Thus, despite the increased $A\beta_{42}/p75^{NTR}$-stimulated progenitor cell proliferation, neurogenesis is not actually increased because fewer progenitor cells survive in the lack of Ach and cilial SSTR3 receptor signalling essential for neuroblasts maturation and *de novo* memory encoding (65) is silenced by the absence of SST in AD brains (66,84).

In this context, a mention is deserved by the Leptin-induced signalling from Leptin b-receptors located in the cell (not primary cilium) membrane, which may also stimulate, via the Shh Smo and the release of cilium-located Gli-A nuclear transcription factor, the primary cilium-dependent proliferation of transit-amplifying progenitors in the dentate *gyrus* of the adult hippocampal formation (85). It follows that daily doses of Leptin might halt AD development if given perhaps in the pre-MCI or MCI stage of the ailment (86).

However, some caution is at present warranted. These tiny primary cilia are a technical challenge to isolate and directly analyse. In addition, we presently do not know whether human dentate gyral granule cells are ciliated or whether human neuroblast maturation and integration into the granule cell layer of SVZ are also driven from primary cilia. On an encouraging note, we have indeed found ciliated cells in samples of hippocampi from octogenarian normal and AD humans and in phenotypically normal astrocytes isolated from adult human cerebral cortices (82).

5. Conclusions

It is quite clear from the foregoing discussion that AD will not be understood by only considering neurons (3,4) and microglia (87). We must take into account the intimate collaboration between neurons and their astrocyte cradlers and trans-network communications. In other words we must pay serious attention to the astrocytes' roles in AD. Moreover, at the subcellular level, important protagonists are emerging such as primary cilia and receptors such as the $p75^{NTR}$ and the CaSR (88), as their interactions with Aβs can modulate or alter fundamental cellular functions like $A\beta_{42}$, NO, VEGF-A and proinflammatory cytokine production and release, proliferative responses and/or damage and malfunctioning of cerebral blood vessels, neurogenesis and cell death. Although as for now the AD picture seems to be more intricate than ever, we hope that these and other new acquisitions on the pathophysiologic mechanisms of this ailment will help pave the way to novel, hopefully effective therapeutic approaches.

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