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INVITED REVIEW

Antagonizing amyloid-β/calcium-sensing receptor signaling in human astrocytes and neurons: a key to halt Alzheimer's disease progression?

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Abstract

Astrocytes' roles in late-onset Alzheimer's disease (LOAD) promotion are important, since they survive soluble or fibrillar amyloid-β peptides (Aβs) neurotoxic effects, undergo alterations of intracellular and intercellular Ca^{2+} signaling and gliotransmitters release *via* the Aβ/α7-nAChR (α7-nicotinic acetylcholine receptor) signaling, and overproduce/oversecrete newly synthesized $Aβ_{42}$ oligomers, NO, and VEGF-A *via* the Aβ/CaSR (calcium-sensing receptor) signaling. Recently, it was suggested that the NMDAR (N-methyl-D-aspartate receptor) inhibitor nitromemantine would block the synapse-destroying effects of Aβ/α7-nAChR signaling. Yet, this and the progressive extracellular accrual and spreading of $Aβ_{42}$ oligomers would be stopped well upstream by NPS 2143, an allosteric CaSR antagonist (calcilytic).

***Key Words:** Alzheimer's disease; amyloid-β; astrocytes; Ca^{2+}; calcilytic; calcium-sensing receptor; nitromemantine; NPS 2143; α7-nicotinic acetylcholine receptor*

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Late-onset Alzheimer's disease (LOAD): a brief introduction

LOAD, the commonest sporadic old-age dementia affecting tens of millions people worldwide starts in layer II neurons of lateral entorhinal cortex, whence it spreads to dentate *gyrus* and CA3 field and thence to higher cortical areas engaged in cognitive functions. LOAD neuropathology's major hallmarks are astrogliosis, amyloid or senile plaques, and neurofibrillary tangles (NFTs). The initial asymptomatic period of LOAD's course lasts ~20–30 years and its detection is hampered by the present lack of specific markers bar the higher LOAD risk posed by having one or two *APOEε4* alleles. The amnestic mild cognitive impairment phase (aMCI; ~2–4 years) presents with amnesias and an intensified BOLD fMRI signal in the dentate *gyrus* and CA3 field. Ability to learn and cope, memory, and cognition are progressively lost in the full-blown phase (6–12 years). As things stand, aMCI is the ideal junction to start a cognition-preserving therapy.

Neurons are not alone in promoting LOAD

Given the marvels it performs, the human brain is the most complex and least understood living "machine". Novel structural and cytological features of cerebrocortical neurons and glial cell types keep emerging and increasing our understanding of the mechanisms that underlie neurodegenerative diseases. Here we shall focus on the roles of glia, particularly astrocytes, in LOAD.

Astrocytes

The very complex and enticing features of astrocytes have been recently reviewed in detail (Verkhratsky et al., 2010; Lee et al., 2014). Here we shall address ourselves to the traits particularly relevant to LOAD. As compared to other mammals, human cortical astrocytes have increased in numbers and size, and are endowed with hundreds of primary processes that branch according to very complex patterns and with their terminals cover "naked" portions of neuronal plasma membranes, partake in a multitude of "tripartite" synapses (~2.0×10^6/astrocyte) of their "client" neurons, and come in contact with adjacent vessels. Moreover, novel polarized and interlaminar astrocyte subtypes that have appeared in humans functionally integrate the layers of the cerebral cortex. Unlike neurons, astrocytes cannot generate propagating action potentials but avail themselves of Ca^{2+} signals of two kinds–intracellular Ca^{2+} transients and intercellular Ca^{2+} waves that rapidly spread through their spatially limited networks of gap–junctionally interconnected peripheral processes. Ca^{2+} signals are made possible by (i) an endoplasmic reticulum (ER) acting as a Ca^{2+}-accruing and Ca^{2+}-releasing stockpile *via*, respectively, its sarco(endo)plasmic reticulum ATPase (SERCA) transport and its ryanodine receptors (RyRs) and inositol 1,4,5-triphospate ($InsP_3$]-receptor channels, and (ii) plasma membrane and intracellular membranes Ca^{2+} channels. Astrocytes' intracellular Ca^{2+} transients and intercellular Ca^{2+} waves, which reveal astrocytes' activation, are responsible for the local and long distance release

of gliotransmitters like glutamate, ATP, D-serine, GABA and taurine. In turn, gliotransmitters modulate astrocyte-astrocyte and astrocyte-neuron signaling (further details and refs. in Verkhratsky et al. (2010) and Lee et al. (2014)). In addition, astrocytes oversee neurons' metabolic needs by acting as bridges functionally integrating into neurovascular units their "client" neurons and the adjacent vessels, with which astrocytes establish contacts *via* the endfeet of their perivascular processes. Thus, any ATP spent during neuronal firing activity is restored *via* an increased influx of oxygen and nutrients brought up by a local vasodilatation. Such changes in local blood flow are made possible because neurotransmitters like glutamate released at active synapses bind astrocytes' specific receptors and activate their intracellular Ca^{2+} signaling driving the exocytotic release of vasoactive substances from the vessel-contacting endfeet. Glutamate released from synapses into the extracellular space is mostly (~80%) taken up by the astrocytes *via* high affinity, Na^+-dependent, GTL-1 and GLAST transporters, which prevents its excitotoxicity. Next, the glutamate is converted to glutamine that is handed over to neurons to be converted again into glutamate and stored inside synaptic vesicles. The astrocytes' glutamate transport is coupled with a Na^+ influx that stimulates glycolysis and the synthesis of lactate, which is next handed over to neurons. The intracellular accrual of Na^+ is cleared up by a Na^+-Ca^{2+}-exchanger (reviewed by Verkhratsky et al. (2010)). In addition, astrocytes express receptors for other neurotransmitters (*e.g.*, purines, GABA, *etc.*) and, of special relevance, N-methyl-D-aspartate receptors (NMDARs). A further aspect of astrocytes' control of brain homeostasis is their control of extracellular ions (particularly K^+) concentrations, pH, and water (reviewed in Verkhratsky et al. (2010)). The features we have briefly recalled here have nurtured the hypothesis that human astrocytes partake, alongside with neurons, in the processes underlying learning, memory, and cognition, which are increasingly lost as LOAD develops.

Until recently the view has prevailed that, like in other neurodegenerative ailments, neurons play a central role in LOAD progression. In fact, neurons produce the main LOAD neurotoxic drivers, *i.e.*, amyloid-β peptides (Aβs) and hyperphosphorylated (p)-Tau proteins, and in the process in creasingly lose their synaptic connections. The upshot is a growing impairment of learning, memory, and cognition. Within this view, astrocytes' roles were limited to partaking in reactive inflammation, and in scavenging exogenously accrued Aβs and cellular debris (reviewed in Li et al. (2011) and Dal Prà et al. (2014b)). However, mounting lines of evidence indicate that, as the keeper of brain homeostasis, neuroglia has the potential to impact on the course and end result of both acute (*e.g.*, ischemic) and chronic (*e.g.*, neurodegenerative) brain diseases. But astrocytes closely team with neurons, and therefore are involved from the very first stages in LOAD neuropathology development (Dal Prà et al., 2014a, b). Yet, being LOAD in most cases such a long-lasting disease, astrocytes' responses can vary with brain site and actual stage of the illness. Studies in 3xTg-AD model mice, which harbor the APP_{Swe}/$PSEN1_{M146V}$/Tau_{P301L} mutations, have shown that in the hippocampal CA1 field and dentate gyrus astrocytes undergo an early atrophy embodied by a markedly reduced arborization of their processes coupled with the loss of tripartite synapses (reviewed in Verkhratsky et al. (2010)). At later LOAD stages a hypertrophic and hyperplastic astrogliosis appears particularly around the fibrillar Aβ plaques—a response probably induced by stressing factors like Aβ aggregates, extracellularly accrued glutamate and/or K^+, oxidative stress, folate deficit, and/or dislipidemias (reviewed in Li et al. (2011)). The astrogliotic cells exhibit cytoskeletal alterations (*e.g.*, glial fibrillary acidic protein [GFAP] overexpression, hyperpolymerized actin), insert their processes into the Aβ plaques, and make and release a surplus of cytokines and chemokines like TNF-α, IL-1β, IL-6, S100β, and interferon (IFN)-γ-inducible protein-10 (IP-10) thereby attracting leukocytes across the blood-brain barrier (BBB) and sustaining a chronic brain neuroinflammation (reviewed in Li et al. (2011) and Lee et al. (2014)). Moreover, the involved astrocytes express a surplus of amyloid precursor protein (APP) and of β-site APP-cleaving enzyme 1 (BACE1/β-secretase), which would lead together with the γ-secretase to an overproduction of Aβs and APPβ in several transgenic AD-model mice (reviewed in Verkhratski et al. (2010)). Hitherto, the APPβ role(s) is (are) not understood. On the other hand, Aβs are a set of peptides of x-43 amino acids, the most common forms of which are the normally 10-fold more abundant $Aβ_{40}$, and the less represented $Aβ_{42}$ peptides.

$Aβ_{42}$ is nontoxic as a monomer, but becomes neurotoxic when aggregates into soluble oligomers of increasing sizes ultimately generating insoluble fibrillar polymers that cluster in plaques. According to the currently prevailing view, the hardly detectable $Aβ_{42}$ oligomers are the first main drivers of LOAD progression to appear (reviewed in Dal Prà et al. (2014a)). Another common but still to be validated view (Armato et al., 2013; Lee et al., 2014) holds that in the normal brain BACE1/β-secretase is expressed solely by neurons; astrocytes express it only when exposed to stressful insults. However, at variance with rodents, proliferatively quiescent cultured but otherwise untreated normal functioning adult human astrocytes (NAHAs) express quite low basal levels of BACE1/β-secretase and β-secretase activity and produce and release quite low basal levels of $Aβ_{42}$; conversely, both enzymatic activities and $Aβ_{42}$ production, intracellular accumulation, and secretion are significantly heightened by an exposure to micromolar concentrations of soluble or fibrillar $Aβ_{25–35}$, an established $Aβ_{42}$ proxy (Armato et al., 2013). In keeping with this, astrocytes from the entorhinal cortex of LOAD patients were found to accumulate Aβs; however, this Aβs accrual was taken as evidence that astrocytes are able to take up and proteolytically degrade exogenously accumulating Aβs (reviewed in Lee et al. (2011)). Conversely, in the $Aβ_{25–35}$-exposed NAHAs, most of the intracellularly accrued Aβs are endogenous $Aβ_{42}$ and $Aβ_{40}$ moieties, the amount of the engulfed exogenous $Aβ_{25–35}$ being rather scanty (Armato et al., 2013 and unpublished results). An exposure to

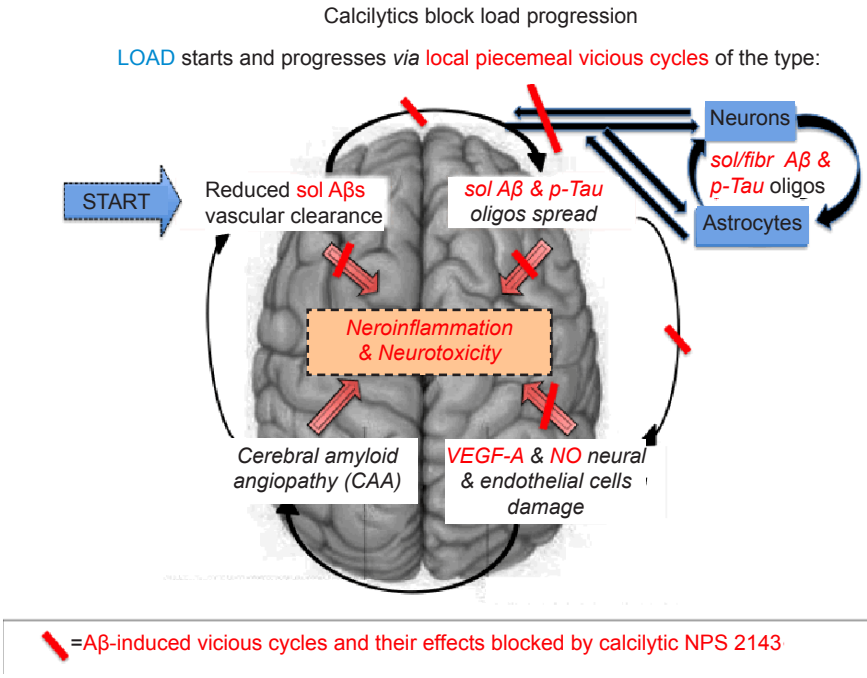

Figure 1 A series of self-promoting vicious cycles triggered by Aβ/CaSR signaling mediates the oversecretion of endogenously produced $Aβ_{42}$ oligomers from ever increasing numbers of human cortical neurons and astrocytes and of NO and VEGF-A from the latter.
Such neurotoxins drive the slow yet progressive dissemination of LOAD neuropathology to cognition-related areas of the cerebral cortex. These harmful effects concurrently causing the death of increasing numbers of cortical neurons are utterly suppressed by allosteric CaSR antagonist NPS 2143.Therefore, calcilytics could halt LOAD progression (Dal Prà et al., 2014a, b, c). Sol: Soluble; fibr: fibrillar; Aβ: amyloid-β peptide; CaSR: calcium-sensing receptor; VEGF: vascular endothelial growth factor; NO: nitric oxide: p-Tau: hyperphosphorylated Tau protein; oligos: oligomers.

combined cytokines (TNF-α plus IFN-γ and/or IL-1β) also increases the production and release of Aβs from cultured adult human astrocytes (Armato et al., 2013). In contrast, accruals of Aβs have been rarely observed within the astrocytes of AD-model mice: but this could be due to rather high intracellular digestion and/or secretion rates rather than to a lacking or insignificant *de novo* synthesis of the $Aβ_{42}$ oligomers. In fact, primary astrocytes isolated from the cerebral cortex of neonatal mouse pups once exposed to a TNF-α + IFN-γ combination or soluble or fibrillar $Aβ_{42}$ exhibited increases in BACE1 activity, APP levels, and endogenous $Aβ_{40}$ secretion suggesting that a feed-forward mechanisms would drive Aβ production in rodent astrocytes under neuroinflammatory conditions (reviewed in Armato et al. (2013)).

Aβs, astrocyte receptors, and Ca^{2+} signaling: a multifaceted story

As mentioned above, the primary LOAD neurotoxic drivers are Aβ oligomers accruing at pathological (nanomolar to micromolar) concentrations within the brain tissue. Part of their complex mechanisms of action are based on the ability to bind several plasma membrane receptors expressed by both astrocytes and neurons—namely the receptors for ApoE, insulin, NMDA, cellular prion protein, advanced glycation end products, and the p75 neurotropin receptor, frizzled receptor, formylpeptide receptor-like 1, α-7-nicotinic acetylcholine receptor (α-7-nAChR) and calcium-sensing receptor (CaSR) (specific refs. in Armato et al. (2013)). Here, we shall focus our discussion on the α-7-nAChR and the CaSR interactions with extracellular Aβs.

An important yet hitherto relatively neglected issue is that at physiological picomolar levels Aβ monomers are devoid of any toxicity, being continuously produced and released at active synapses. Thus, physiologically by binding astrocytes' α7-nAChRs with picomolar affinity Aβ monomers enhance spontaneous intracellular Ca^{2+} signals, but never trigger intercellular Ca^{2+} wave signals (Lee et al., 2014). Hence, picomolar Aβ/α7-nAChR signaling could induce the release of gliotransmitters like ATP, D-serine, and glutamate from the activated astrocytes as well as trigger Ca^{2+} influxes leading to the release of glutamate from neurons. In turn, extracellularly secreted glutamate binds astrocytes' glutamate receptors eliciting further intracellular Ca^{2+} transients. Therefore, at picomolar concentrations $Aβ_{42}$ monomers partake in the reciprocal modulation of physiological neuron-astrocyte signaling that would enhance long-term potentiation (LTP) and memory (Lee et al., 2014).

Although at pathological (nanomolar to micromolar) concentrations $Aβ_{40}$ oligomers may inhibit or not change astrocytes' intracellular Ca^{2+} transients and hence α7-nAChRs-mediated glutamate release, Talantova et al. (2013) have reported that at low micromolar concentrations *via* α7-nAChR signaling both $Aβ_{42}$ and $Aβ_{25–35}$ release glutamate from purified cultures of rat and human astrocytes; in turn, the secreted glutamate activates *extrasynaptic* NMDARs on neurons triggering increases in NO, p-Tau oligomers, and caspase-3 activity that destroy the synaptic spines, the first step of the road leading to neuronal death. In addition, in the astrocytes of human LOAD patients there occurs a deregulated α7-nAChRs signaling due to a strongly heightened expression of the α7 receptor subunit that is coupled with abnormally intense Ca^{2+} influxes and intracellular Ca^{2+} signals, altogether indicating a deeply altered activation of the astrocytes conducive to neurotoxicity. Moreover, intercellular Ca^{2+} waves have been detected in the astrocyte networks surrounding the Aβ plaques of *in vivo* patients–waves Aβ monomers at picomolar concentrations never trigger (Lee et al., 2014). According to other reports, cultured astrocytes exhibited after an exposure to Aβ a lessened expression and activity of glutamate transporters and a decreased glutamate

uptake, whereas in aged AD-model mice neocortical astrocytes displayed a heightened expression of AMPA/kainate glutamate receptors and of glutamate transporters. Though discrepant, altogether these findings indicate that significant alterations of the astrocyte-neuron glutamate-glutamine shuttle, glutamatergic transmission, and local glutamate toxicity occur in LOAD (reviewed in Verkhratsky et al. (2010), Li et al. (2011), and Lee et al. (2014)).

Oligodendrocytes

Besides receiving synaptic inputs, NG-2 glial cells are progenitors of protoplasmic astrocytes, oligodendrocytes, and maybe neurons. Exposure to neurotoxic levels of Aβs inactivates the Wnt signaling pathway preventing the differentiation of NG-2 cells into these cell types (Peng et al. 2014). Human oligodendrocytes, though minimally turning over after five years of age, rapidly modulate myelin production according to ongoing needs, *e.g.*, learning activities, thereby promoting neural plasticity. Hitherto, oligodendrocytes' roles in LOAD progression have been largely neglected. Roth et al. (2005) reported that Aβs elicit oligodendrocytes' death and myelin sheaths degeneration in the hippocampus. The breaking down sheaths release myelin basic protein (MBP), which in turn can trigger a neurotoxic discharge of nitric oxide (NO) from cultured cortical NAHAs (reviewed in Armato et al. (2013)).

Microglia

Conversely, any search in PubMed will show that microglia's activation and its consequences on LOAD's course have been subjected to intense investigations. Both microglia and astrocytes trigger the chronic inflammation going on in LOAD brains. Notably, microglia's proinflammatory role may even be less relevant than astrocytes' due to microglia's lower numbers and shorter-lasting activation (reviewed in Lee et al. (2011)). The proinflammatory cytokines (*e.g.*, IL-1β) secreted by the activated microglia bind to astrocytes' receptors, thereby increasing their release of Aβs and of cytokines (*e.g.*, S100β). In turn, these compounds sustain and/or intensify the activation of the microglia and the release of neurotoxic Aβs from astrocytes and neurons, thereby further harming the neurons (reviewed in Lee et al. (2011)). These self-maintaining and spreading vicious cycles can help promote the progression of LOAD neuropathology.

Cerebral vessels

Furthermore, the interactions within the neurovascular units between astrocytes and cerebral vessels are also relevant to LOAD progression and the onset of cerebral amyloid angiopathy (CAA), because the micromolar Aβ-exposed and activated astrocytes release surpluses of Aβ42 oligomers, VEGF-A, and NO (Armato et al., 2013; Dal Prà et al., 2014c) onto the vessels' walls. These agents cause cellular dysfunctions and a defective angiogenesis *in vivo* that alter the BBB permeability and worsen the noxious effects of LOAD's neurotoxic drivers accelerating the progression of the neuropathology and in parallel of cognition deficits (**Figure 1**).

In conclusion, the interactive dysfunctional responses of all brain-resident cell types and not of neurons only contribute to the promotion of LOAD neuropathology (Peng et al., 2014).

Novel neuroprotective actions of calcilytics

As stated at the beginning, there is still relatively little knowledge and agreement about the mechanisms promoting LOAD onset and progression, and in relation to this an early diagnosis is hard to achieve in the absence of specific markers, and no effective therapy is presently available. Hitherto, animal models have allowed significant strides into the mechanistic understanding, yet not the therapeutics of LOAD. Conceivably, work using as experimental models untransformed human neural cells cultured in vitro should help clarify the picture. To this aim, we have been using proliferatively quiescent NAHAs with a locked-in phenotype, isolated from anonymized surgical tiny temporal cortex leftovers and set into pure cultures.

Initially, we showed that a mixture of IL-1β, TNF-α, and IFN-γ-the "cytokine mixture (CM)-trio" released from activated microglia-induced NAHAs' NOS-2 mRNA expression *via* a p38 MAPK activity surge and the *de novo* synthesis of NOS-2 protein. But the dimerization and activation of the newly synthesized NOS-2 protein needed a MEK/ERK-dependent tetrahydrobiopterin (BH4) synthesis by the co-induced GTP cyclohydrolase-1 (GCH1). Interestingly, GCH1 activation was totally prevented by adding NPS 89686, a selective CaSR antagonist ("calcilytic") (Armato et al., 2013). Parenthetically, the CaSR is a member of family 3 of G protein-coupled receptors (GPCRs), also named seven transmembrane spanning receptors (7TMRs), and plays crucial regulatory roles in Ca^{2+} homeostasis and cellular signaling. The CaSR is expressed by all brain-resident cell types, and its recently reviewed (Armato et al., 2013; Dal Prà et al., 2014b) complex features will not be mentioned here, save recalling that it binds on orthosteric (calcium ion) and several allosteric positively charged ligands and is endowed with a panoply of distinct and modulable G-protein mediated intracellular signaling pathways. Yet, at that time what linked the CM-trio to a CaSR-triggered MEK/ERK signaling that activated GCH1 and hence NOS-2 in NAHAs was unclear. Concurrently, we found that both MBP and soluble micromolar $A\beta_{40}$, each by itself and also in synergism with the CM-trio, intensely induced and activated NOS-2 in NAHAs (reviewed in Armato et al. (2013)). These findings suggested a potential mediation of the CM trio-elicited NO overproduction *via* an Aβ/CaSR signaling in NAHAS. In this regard, various authors had shown that (i) combinations of two cytokines induce NAHAS to release Aβs, and (ii) Aβs specifically bind the CaSR and activate its signaling (Armato et al., 2013; Dal Prà et al., 2014c). Hence, a crucial question concerning LOAD promotion arose: would the Aβ/CaSR signaling coax human astrocytes and perhaps neurons to overproduce and oversecrete first endogenous $A\beta_{42}$ and later NO? Such a CaSR signaling-mediated Aβ-self-inducing mechanism could start an intra-brain "contagion-like"

spread of $A\beta_{42}$ oligomers by recruiting ever increasing numbers of astrocytes and neurons to spew out further surpluses of $A\beta_{42}$ oligomers, which would in due course foster the formation and harmful activity of p-Tau oligomers and hence the dissemination of LOAD neuropathology (reviewed in Dal Prà et al. (2014a)) (**Figure 1**). The demonstrations that intra-brain spreading amyloidoses can be induced by Aβs injected into the brains of APP transgenic mice, wild-type rats, and marmosets (reviewed in Dal Prà et al. (2014a)) supported the feasibility of our hypothesis and enticed us to test it on cortical NAHAs and human postnatal HCN-1A neurons. First, we found that widely set apart micro-aggregates of biotinylated fibrillar $A\beta_{25–35}$ carefully placed on the astrocytes' monolayer surface caused all the NAHAs, and not only those bordering on the $A\beta_{25–35}$ micro-heaps, to accrue and oversecrete endogenous $A\beta_{42}$ oligomers within 24–48-hours. Contrariwise, no endogenous $A\beta_{42}$ above basal levels accumulated within or was secreted by the untreated or reverse mer $A\beta_{35–25}$-treated NAHAs. Remarkably, at a low micromolar concentration exogenous *soluble* $A\beta_{25–35}$ too powerfully increased the accrual and *secretion* of endogenous $A\beta_{42}$ oligomers from NAHAs. Such findings indicated that Aβ-exposed astrocytes could significantly increase the brain's Aβs burden and contribute to intracerebral LOAD spreading (Armato et al., 2013). Next, we determined whether the micromolar Aβ/CaSR signaling did indeed mediate this "*exogenous-Aβs-begetting-endogenous-Aβs*" in NAHAs by using highly selective and specific modulators of the CaSR's sensitivity to Ca^{2+}. Thus, an allosteric CaSR agonist ("*calcimimetic*"), NPS R-568, increased the NAHAs' *endogenous* $A\beta_{42}$ secretion thereby demonstrating the involvement of the receptor in this process. On the other hand, an allosteric CaSR *antagonist* ("calcilytic"), NPS 2143, given alone did not change the NAHAs' basal turnover of endogenous $A\beta_{42}$. Yet, a short-lasting pretreatment with NPS 2143 totally quashed the NAHAs' intracellular accrual and surplus secretion of endogenous $A\beta_{42}$ oligomers otherwise elicited by exogenous $A\beta_{25–35}$ given by itself. Consequently, NPS 2143 kept the extracellular $A\beta_{42}$/$A\beta_{40}$ ratio values within the normal, *i.e.* non toxic range, and concurrently quickly and persistently curbed NAHAs' total CaSR protein levels thereby desensitizing them to exogenous Aβs. Therefore, a calcilytic could effectively suppress the Aβ/CaSR triggered overproduction and overrelease of endogenous $A\beta_{42}$ oligomers and, in parallel, of NO from NAHAs (Armato et al., 2013). To strengthen our "*Aβs-contagion*" or Aβs self-induction hypothesis of LOAD dissemination (Dal Prà et al., 2014a, b) we assessed the behavior of similarly challenged *in vitro* cortical human postnatal HCN-1A neurons. Exogenous fibrillar or soluble $A\beta_{25–35}$ remarkably increased the somatic, dendritic, axonal, and secreted amounts of *endogenous* $A\beta_{42}$ in the HCN-1A neurons. Adding NPS 2143 prior to fibrillar or soluble $A\beta_{25–35}$ strikingly left the dendritic, axonal, and Golgi/endosomal compartments devoid of endogenous $A\beta_{42}$ while keeping the $A\beta_{42}$ secretion rates and extracellular $A\beta_{42}/A\beta_{40}$ ratio values at basal levels. Therefore, Aβ/CaSR signaling induced an alike *calcilytic-inhibitable* endogenous $A\beta_{42}$ oversecretion from both human cortical astrocytes and neurons (Armato et al., 2013). Next, we tackled the "hot" issue of human neurons' survival under the Aβs' challenge. Thus, we found that NAHAs' viability was not affected by micromolar $A\beta_{25–35}$ plus/minus NPS 2143. Conversely, micromolar $A\beta_{25–35}$-exposed HCN-1A neurons slowly yet progressively died. But, adding calcilytic NPS 2143 fully preserved $A\beta_{25–35}$-treated neurons' viability, while NPS 2143 given alone did not hamper it. Consequently, by keeping neurons alive and functioning a calcilytic treatment would potentially safeguard human cognitive capabilities (Armato et al., 2013). Meanwhile, we identified another LOAD promotion-relevant Aβ/CaSR-mediated effect on NAHAs. Fibrillar $A\beta_{1–42}$ or $A\beta_{25–35}$ intensely stimulated VEGF-A production/secretion from normoxic NAHAs (recall that VEGF-A is neurotrophic at low, but neurotoxic at high concentrations). Again, an Aβ/CaSR-signaling was involved as NPS 2143 wholly suppressed both $A\beta_{25–35}$- and $A\beta_{1–42}$-elicited VEGF-A overreleases from the astrocytes. The ability of calcimimetic NPS R-568 to stimulate NAHAs' VEGF-A oversecretion further authenticated the CaSR's involvement in such a process (Dal Prà et al., 2014c).

Additionally, Kim et al., (2014) reported that the two calcilytics we had been using–NPS 2143 and NPS 89696–prevented the neurons' death due to CaSR overexpression and $GABA_BR1$ downregulation in animal models of global or focal brain ischemia. Therefore, both Kim et al.'s and our group's findings stress that CaSR's pathological signaling following an exposure either to Aβ oligomers or to brain ischemia underlies a *calcilytic-preventable loss* of neurons. Therefore, so far calcilytics' neuroprotective power has been proven in two distinct harmful settings!

Calcilytics as anti-LOAD drugs: pros beat cons

Conceivably, if taken early enough, *e.g.*, during aMCI or earlier when it will be feasible, calcilytics could stop (or slow) LOAD dissemination *via* a CaSR-mediated Aβ self-induction in both neurons and astrocytes (Dal Prà et al., 2014a, b, c). By keeping extracellular $A\beta_{42}/A\beta_{40}$ values within the physiological range, calcilytics would (i) safeguard/reinstate the Wnt signaling needed for neurogenesis in the dentate gyrus subgranular zone and hence the patient's ability to store and retrieve memories; and (ii) maintain the structural/functional integrity of cognition-crucial higher cortical areas and hence the person's coping ability, life quality, and life expectancy. Unluckily enough, an "*original sin*" has tarnished calcilytics —by overstimulating a parathyroid hormone release that increased both osteogenesis and osteolysis they have hitherto failed as anti-osteoporosis drugs. Moreover, calcilytics' potential side effects—*e.g.*, hypertension in rats, mild hyperparathyroidism in humans—have nearly removed them from the beneficial drugs inventory. Accordingly, proposing calcilytics as anti-LOAD drugs might sound bizarre. However, there are several valid reasons why anti-calcilytics stances should be seriously reconsidered. As drugs, calcilytics are easily administered and cross the BBB (our unpublished findings). And, besides blocking the multiple neurotoxic effects of Aβs, including Aβs-and ischemia-induced neuronal deaths, calcilytics attenuate the noxious consequences

of CaSR's gain-of-function mutations (refs. in Dal Prà et al. (2014b)) and override pulmonary artery hypertension (Yamamura, 2014). Therefore, the anti-LOAD effects of calcilytics should be thoroughly assessed in AD-model animals and humans. Moreover, calcilytics' side effects must be weighed against the harsh truth that full-blown LOAD is inexorably lethal. Hence, as for anticancer chemotherapeutics, the use of calcilytics should be enabled, since their side effects, manageable by current medical wisdom, are a small price against stopping/preventing LOAD progression.

Conclusion and perspectives

In summary, the physiology and LOAD pathophysiology of astrocytes are extremely complex and differ according to the animal species, age, brain site, and the development stage of the disease. However, data about these topics specifically pertaining to untransformed human adult astrocytes and neurons are beginning to accrue. Seemingly, the most important fact to keep in mind is that, at variance with neurons, the human astrocytes can withstand the neurotoxic onslaught brought about by micromolar soluble or fibrillar Aβs. Hence, they undergo a set of reactive functional changes involving an altered intracellular and intercellular Ca^{2+} signaling and gliotransmitters release *via* the Aβ/α7-nAChR signaling and an overproduction/oversecretion of newly synthetized $Aβ_{42}$ oligomers, NO, and VEGF-A *via* the Aβ/CaSR signaling, and the secretion of other toxic factors (*e.g.*, proinflammatory cytokines, chemokines, reactive oxygen species [ROS], *etc.*) presumably *via* Aβs interaction with other receptors and/or non-receptor-mediated Aβs mechanisms. Altogether these complex responses indicate that human astrocytes play quite a significant role on LOAD's promotion. They might even release a high proportion of N-terminally truncated $Aβ_{3–42}$ that is converted into the most toxic pyroglutamyl-$Aβ_{3–42}$. Clearly, the path to achieve LOAD's cure is still long and deceitful but not without some glimmer of hope. Recently, Talantova et al. (2013) have proposed the NMDAR inhibitor nitromemantine, an improved memantine derivative, to block the synapse-destroying effects of pathological Aβ/α7-nAChR signaling and thus preserve cognition. Yet, this and the broad extracellular accrual and spreading of neurotoxic $Aβ_{42}$ oligomers could be stopped well upstream by administering an allosteric CaSR antagonist or calcilytic (Armato et al., 2013; Dal Prà et al., 2014a, b, c). Overall, in these studies science goes along with purposes of humanitarian welfare and with the urgent need of preventing the already huge socioeconomic burden of LOAD patients' care from becoming unbearable.

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