

## New Insights on Astroglial Connexins as a Therapeutic Target for Alzheimer's Disease

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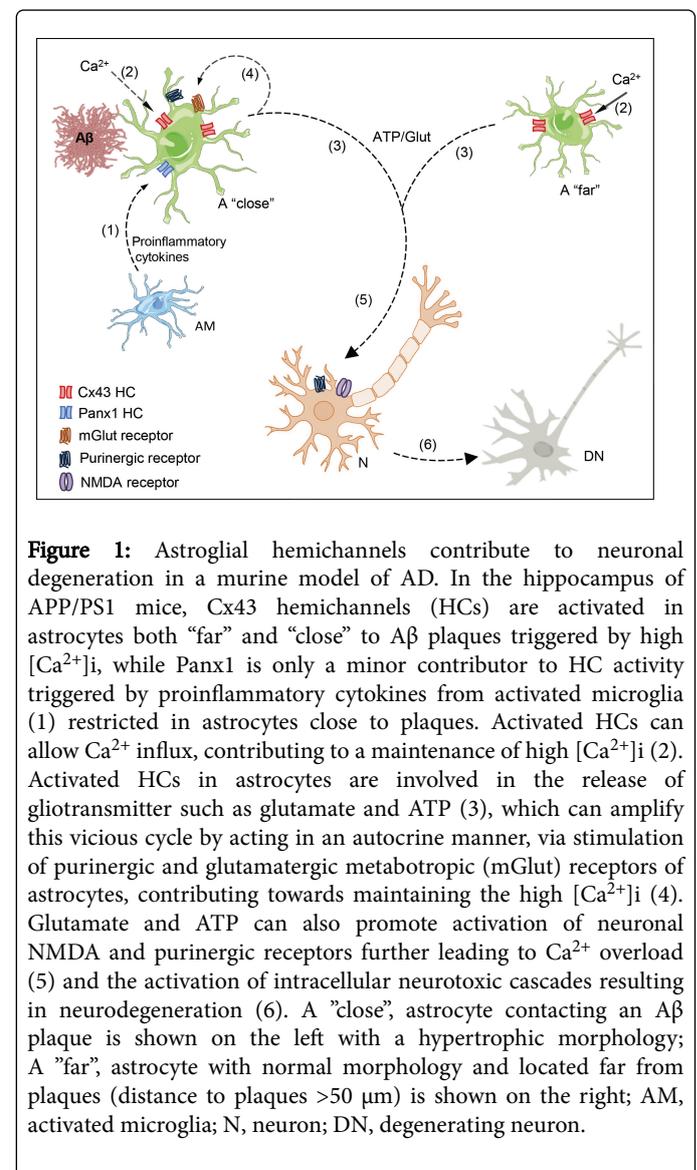
### Commentary

Alzheimer's disease (AD) is the leading cause of dementia in the elderly and characterized by progressive memory loss, behavioral deficits and significant personality alterations [1,2]. The histopathological hallmarks of AD include A $\beta$  plaques, neurofibrillary tangles, neuronal death and synapse loss. One significant feature of A $\beta$  accumulation is the strong reactive gliosis associated with the A $\beta$  plaques themselves [3,4], however, understanding of the properties and functions of astrocytes in AD has only begun to emerge in recent years.

In the brain, astrocytes possess the highest levels of connexin (Cx) expression, with Cx43 and Cx30 being the most abundant [5,6]. Cxs are the proteins that form gap junction channels (GJCs) and hemichannels (HCs). Besides Cxs, HCs can be formed by another family of functionally related proteins, pannexins (Panxs), which share the same transmembrane topology as Cxs but have divergent primary sequences [7]. Cxs and Panxs oligomerize into hexamers that isolate a large non-selective pore in the membrane allowing for the diffusion of small molecules (e.g. ions, small signaling molecules and metabolic substrates) between the cell cytoplasm and the extracellular medium. In addition, most Cx HCs dock head-to-head between adjacent cells to form intercellular channels that constitute GJCs, while endogenously expressed Panx HCs do not [8,9]. In the brain, astrocytes provide trophic and metabolic support to neurons throughout the astrocytic network via gap junction communication which comprise of over several hundreds of astrocytes [10].

In brain pathologies, for instance in AD, reactive astrogliosis has been associated with changes in the expression and function of connexins. Increased expression of Cxs has been found in astrocytes that are in contact with amyloid plaques *in vivo* within the brains of AD patients and also in AD animal models. The most prominent indication of connexin changes in Alzheimer's disease is that increased immunoreactivity of Cx43 has been found at A $\beta$  plaque levels in the post-mortem brains of AD patients, which coincides with the presence of gap junctions between astrocytic processes that are adjacent to dystrophic neuronal processes that are present in plaque areas at the ultrastructural level [11]. This Cx43 puncta enrichment has also been found within cadaveric brain sections from AD patients and is detected intermingled with strongly GFAP-positive astrocytic processes that infiltrate A $\beta$  plaques [12]. These features have also been shown to be present in Cx30, though to a lower extent. This is also the case in AD animal models. In a murine model of familial AD (APP<sup>Swe</sup>/PS1<sup>dE9</sup>; or termed APP/PS1), these transgenic mice exhibit a variety of changes, including an increase in Cx43 or Cx30 immunoreactivity at A $\beta$  plaque levels in the hippocampus and cortex of APP/PS1 mice older than 4 months. These connexin immunoreactivities have been found to be concentrated in bright and

large puncta at astrocytic processes that infiltrate the plaque core, and are encircled by dystrophic neurites [12,13].



On the other hand, the channel functions of Cxs including gap junction channels and hemichannels can also be modified in AD. Under normal circumstances, gap junctional communication is relatively high in astrocytes. Thus far, published data from AD mouse

models demonstrate that an enhancement or level-wise maintenance of intercellular communication in astroglial networks is a feature of these mice. Therefore, gap junction channels are likely to be functional in these mice, allowing the diffusion of various substances that may play a variety of roles to distant sites. For instance, astrocytic gap junction channels in the cortex are involved in the propagation of intercellular calcium waves (ICWs) which influence neuroglial interactions, and hence modify neuronal activity and/or neuronal survival. This effect is induced through  $\text{Ca}^{2+}$  dependent mechanisms that are part of  $\text{K}^+$  homeostasis [14], intercellular metabolic wave propagation [15], gliotransmission [16] and blood flow control at the glio-vascular surface [17]. In our recent studies, we compared GJCs in hippocampal astrocytes from 9-month-old APP/PS1 mice with age-matched control using a fluorescence recovery after photobleaching (FRAP) method in acute brain slices loaded with Sulforhodamine 101, which is a gap-junction permanent dye with selective uptake by astrocytes. It has been found that astroglial gap junctional communication is maintained in APP/PS1 mice and is not affected by the reactivity of astrocytes [18]. The investigation of hemichannel function in astrocytes from APP/PS1 mice can be performed by using acute brain slices to understand the respective contribution of connexins and pannexins to hemichannel activity by using pharmacological approaches such as the Ethidium Bromide uptake assay [5]. In recent studies conducted by our group, Cx43 was revealed to be the main hemichannel contributor in all of the astrocytes in hippocampus of APP/PS1 mice, and a minor Panx1 component was also detected in the subpopulation of astrocytes contacting amyloid plaques [18]. This suggests that connexin or pannexin hemichannel activation is regulated by different pathways, and represents the idea that the contribution of Panxs and Cxs towards HC function in astrocytes depends largely on the conditions that trigger their subsequent activation. We revealed that in APP/PS1 mice, Panx1 HCs are triggered by inflammatory cytokines likely released by activated microglia, while the triggering of the Cx HC activation is by the high intracellular calcium ion concentration ( $[\text{Ca}^{2+}]_i$ ) in astrocytes [18].

Activated HCs have been involved in the release of gliotransmitters such as ATP and glutamate as well as chemokines that have deleterious consequences on neurons as reported in murine models of AD [18-20], experimental allergic encephalomyelitis [21], amyotrophic lateral sclerosis [20,22] and neuropathic pain [23,24]. In our study, it has also been demonstrated that activated HCs can allow  $\text{Ca}^{2+}$  influx, contributing to a maintenance of high  $[\text{Ca}^{2+}]_i$ . The release of glutamate and ATP associated with HC activation in astrocytes can also amplify this vicious cycle by acting in an autocrine manner, via stimulation of purinergic and glutamatergic metabotropic receptors of astrocytes, contributing to maintaining high  $[\text{Ca}^{2+}]_i$ . Meanwhile, glutamate and ATP can also promote activation of neuronal NMDA and purinergic receptors, further leading to  $\text{Ca}^{2+}$  overload and the activation of intracellular neurotoxic cascades resulting in neurodegeneration [18]. This is summarized in Figure 1.

Diverse strategies aimed at blocking HC activity using genetic or pharmacological tools have thus been developed [25-28]. Most of them suppress Cx43 expression and/or function, which are considered the main HC constituent in astrocytes [7] by knocking-out Cx43 gene, for instance, using anti-sense tools, antibodies or mimetic peptides. However, all these attempts also impact astroglial gap junctional communication as well, which result in a mechanistically biased interpretation of the observations. Therefore, an approach that blocks only HC function in glial cells may represent a promising tool that tunes down their deleterious effects in brain pathologies. In

neurodegenerative diseases in particular, the design of a therapeutic strategy has to take into account the need for chronic treatment and the use of molecules that are able to pass the blood-brain-barrier. In our work, we have selected a naturally found alkaloid compound, boldine, which is extracted from a Chilean tree, and analyzed its effects on HC activity and gap junctional communication in glial cells in different experimental paradigms: cultured astrocytes and acute brain slices in which HC activation was triggered by pro-inflammatory treatment in astrocytes and microglia, as well as in brain slices of 9-month-old APP/PS1 mice, in which we recently reported that HCs are chronically activated in astrocytes in the hippocampus [18]. We show that boldine was as efficient as carbenoxolone, a HC blocker, in inhibiting HC activity in astrocytes and in microglial cells but had no effect on astroglial gap junctional communication. More interestingly, we also provide evidence that boldine can be administered *in vivo* as a pharmacological tool with similar effects on these two channel functions. The ability of boldine to target glial HC function prompted us to investigate its effect *in vivo* by treating APP/PS1 mice with this drug. Long-term administration of boldine *in vivo* in APP/PS1 mice prevented HC activation, resulting in the reduction of ATP and glutamate release, decreasing astroglial  $[\text{Ca}^{2+}]_i$  to resting levels and the alleviation of neuronal damage in the hippocampus [29]. Consequently, boldine can be a drug treatment that opens the way to designing novel protective molecules that allow the reduction of neuronal damage associated with neurodegenerative situations, in particular amyloid pathology. Nevertheless, we cannot exclude that other functions of boldine, for instance its anti-oxidant properties [30], may also be involved in the alleviation of neuronal damage in AD. More work can be done to examine other small molecular blockers with higher specificity to HCs, given its apparent contribution to disease, which will allow for more in depth studies of hemichannel function within pathological conditions.

The current state of the art demonstrates that a comprehensive study of astroglial connexins in understanding the pathology of neurodegenerative disease like AD is crucial in a greater understanding of these diseases. Astroglial connexins could be a new therapeutic target in the treatment of AD. Such an undertaking will allow us to develop novel strategies towards the development of pharmaceutical tools that can alleviate the great burden of neurodegenerative diseases on society.

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## Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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