Astrocytic clasmatodendrosis, autofluorescent deposits and accumulation of Aβ-peptide? in CA1 hippocampus: possible links with aging-dependent neurodegeneration?

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Aging is a primary risk factor in Alzheimer disease (AD), characterized by β-amyloid (Aβ) burden and alterations of neuron-astrocyte interactions. Clasmatodendrosis, defined as fragmentation of astrocytes projections (APJs), occurs in both AD and normal aging. Despite the key-role played by astrocytes in neuron-astrocyte interaction, little attention has been given to the possible involvement of astrocytic clasmatodendrosis on the accumulation of fibrillary Aβ in neurodegeneration. Thus, our present study was aimed at assessing the possible link between clasmatodendrosis and Aβ-deposition in CA1 hippocampus of aged rats. Using confocal microscopy and multiphoton fluorescence lifetime imaging (FLIM) together with an innovative Phasor analysis, according to Digman (1), we analyzed autofluorescence and immunofluorescence for GFAP, Aβ-peptide and NeuN in CA1 hippocampus of aged and adult rats as controls. In accordance to our previous results (2) we found that APJs were shortened and retracted in CA1 of aged rats, suggesting the occurrence of clasmatodendosis. Using FLIM/Phasor analysis we found that in the hippocampus of aged rats autofluorescence significantly increased and almost completely colocalized with GFAP and Aβ-peptide immunofluorescence. The percentage of Aβ-peptide immunofluorescence that colocalized with autofluorescence was significantly higher in aged than in adult rats. Furthermore, we found two different types of association between GFAP immunofluorescence and autofluorescent Aβ-peptide deposits, possibly due to their different size: a) small Aβ-peptide islets (area 0.05±0.01 μm^2), frequently present on intact APJs, particularly in control rats; b) large accumulations of Aβ-peptide (area >1.94 μm^2), markedly associated with fragmented APJs in aged rats. Using particle analysis software, autofluorescent islets were isolated on the basis of their size. We evaluated the amount and distribution of Aβ-islets in CA1 *Str. pyramidale*, *Str. oriens* and *Str. radiatum.* The amount of Aβ-peptide islets was significantly higher in aged than in adult rats. In aged rats Aβ-islets decreased in *Str. oriens* and *Str. radiatum* and were significantly increased in *Str. pyramidale* in comparison to control rats. Autofluorescent Aβ-peptide deposits were consistently localized on neurons immunostained for NeuN, frequently at interneuron or neuron/astrocyte contact sites. Digital cell subslicing showed the presence of intracellular autofluorescent Aβ-deposits that often adhered to GFAP-positive APJs. These findings suggest that APJs fragments, indicative of clasmatodendrosis, and deposits of Aβ-peptide increase in CA1 hippocampus of aged rats. These features are distinctive phenomena present in the aged rat hippocampus. Direct interaction of APJs fragments and Aβ-peptide deposits with neuron cell bodies may possibly be responsible for decreased neuronal functionality.

1) Digman et al. (2008). *Biophys J* 94: 14-16.
2) Cerbai et al. (2012). *PLoS ONE* 7(9): e45250.