Neurobiology of Aging xxx (2013) 1–9

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Rapid communication

Complex and region-specific changes in astroglial markers in the aging brain

José J. Rodríguez [a,b,*], Chia-Yu Yeh [c], Slavica Terzieva [a,b,d,e], Markel Olabarria [c], Magdalena Kulijewicz-Nawrot [f], Alexei Verkhratsky [a,b,c,**]

[a] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[b] Department of Neurosciences, University of the Basque Country UPV/EHU and CIBERNED, Leioa, Spain
[c] Faculty of Life Sciences, The University of Manchester, Manchester, UK
[d] Laboratory of Neuroendocrinology-Mole Cell Physiology, Institute of Pathophysiology, University of Ljubljana Medical Faculty, Ljubljana, Slovenia
[e] Celica, Biomedical Center, Ljubljana, Slovenia
[f] Institute of Experimental Medicine, ASCR, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form 4 June 2013
Accepted 5 July 2013

Keywords:
Astrocytes
Aging
GFAP
s100β
Glutamine synthetase

ABSTRACT

Morphological aging of astrocytes was investigated in entorhinal cortex (EC), dentate gyrus (DG), and cornu ammonis 1 (CA1) regions of hippocampus of male SV129/C57BL6 mice of different age groups (3, 9, 18, and 24 months). Astroglial profiles were visualized by immunohistochemistry by using glial fibrillary acidic protein (GFAP), glutamine synthetase (GS), and s100β staining; these profiles were imaged using confocal or light microscopy for subsequent morphometric analysis. GFAP-positive profiles in the DG and the CA1 of the hippocampus showed progressive age-dependent hypertrophy, as indicated by an increase in surface, volume, and somata volume at 24 months of age compared with 3-month-old mice. In contrast with the hippocampal regions, aging induced a decrease in GFAP-positive astroglial profiles in the EC: the surface, volume, and cell body volume of astroglial cells at 24 months of age were decreased significantly compared with the 3-month group. The GS-positive astrocytes displayed smaller cellular surface areas at 24 months compared with 3-month-old animals in both areas of hippocampus, whereas GS-positive profiles remained unchanged in the EC of old mice. The morphometry of s100β-immunoreactive profiles revealed substantial increase in the EC, more moderate increase in the DG, and no changes in the CA1 area. Based on the morphological analysis of 3 astroglial markers, we conclude that astrocytes undergo a complex age-dependent remodeling in a brain region–specific manner.

1. Introduction

Astrocytes are the main homeostatic cells of the central nervous system (CNS). Astroglia control brain homeostasis at all levels including, for example, organ homeostasis (astrocytes control the emergence and maintenance of the blood-brain barrier), cellular homeostasis (astroglia/radial glia are stem elements for embryonic and adult neurogenesis), morphological homeostasis (astrocytes define nervous system microarchitecture and control synaptogenesis), molecular homeostasis (astrocytes regulate ion, neurotransmitter, and neurohormone concentrations in the nervous tissue), metabolic homeostasis (astrocytes supply neurones with energy substrate), and defensive homeostasis (represented by astrogliosis) (for recent reviews, see Kimelberg and Nedergaard, 2010; Parpura et al., 2012). Astrocytes also play a central role virtually in all forms of neuropathology, determining, to a large extend, the progression and outcome of neurologic diseases (Giaume et al., 2007; Rodriguez et al., 2009; Verkhratsky et al., 2012).

It is a general consensus that physiological brain aging is not associated with substantial changes in the number of neurones in the most of the brain areas (Toescu and Verkhratsky, 2007; Turlejski and Djavadian, 2002). Much less is known about aging of astroglia; it is almost universally believed that aging is associated with astroglial proliferation and increase in the expression of glial fibrillary acidic protein (GFAP), both considered to be signs of reactive astrogliosis (Cotrina and Nedergaard, 2002; Lynch et al., 2010; Schipper, 1996; Unger, 1998). Morphological analysis of aged astrocytes, however, is mainly confined to hippocampal regions; in other parts of the CNS, both increases (Cotrina and Nedergaard, 2002; Wu et al., 2005) and decreases (Cotrina and Nedergaard, 2002; Lasn et al., 2006; Mansour et al., 2008; Nishimura et al., 1995; Wu et al., 2005) in astroglial density at old ages were documented. Studies of astroglial morphology are

* Corresponding author at: IKERBASQUE, The University of the Basque Country UPV/EHU, Technological Park, Bldg. 205, Floor -1. Laida Bidea, 48170 Zamudio, Bizkaia, Spain. Tel.: +34 946018305; fax: +34 946018289.
** Alternate corresponding author at: Faculty of Life Science, The University of Manchester, Oxford Road, Manchester 13 9PT, UK.
E-mail addresses: j.rodriguez-arellano@ikerbasque.org (J.J. Rodríguez), Alexej.Verkhratsky@manchester.ac.uk (A. Verkhratsky).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.07.002

further complicated by the fact that the most common marker, GFAP, does not faithfully delineate the complex structure of individual astrocytes, which is often characterized by extremely elaborated terminal processes underlying their spongiform appearance (Bushong et al., 2002; Nedergaard et al., 2003). Furthermore, GFAP is not a universal astroglial marker because subpopulations of GFAP-positive astrocytes vary between brain regions with GFAP-positive population accounting for ~80% of cells in the hippocampus and only 15%–20% in the cortex (Kimelberg, 2004).

In addition to cytoskeletal changes, aging also has deep effects on astrocytic functions specifically in glutamate homeostasis such as glutamate uptake and/or expression/activity of glutamate synthetase (GS; Olabarria et al., 2011). However, findings are inconsistent likely because of different experimental designs and methodology (García-Matas et al., 2008; Goss et al., 1991; Gottfried et al., 2002; Wu et al., 2005). Astrocytes are highly heterogeneous in their morphological features and physiological properties; therefore, they may react differently to the aging process. Here, we report differential effects of aging on astroglial profiles labeled with 3 specific markers, GFAP, GS, and astroglial protein s100β studied in the hippocampus and in the entorhinal cortex (EC) of mice.

2. Materials and methods

All animal procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act of 1986 under the license from the Home Office. Experimental techniques have been described in detail elsewhere (Olabarria et al., 2010, 2011; Rodriguez et al., 2008; Yeh et al., 2011). Briefly, experiments were performed on male SV129/C57BL6 mice of different age groups (3, 9, 18, and 24 months; $n = 3–7$), which were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). Mice were perfused through the aortic arch with 3.75% acrolein (25 mL; TAAB, Berkshire, UK) in 2% paraformaldehyde (PFA; Sigma, UK) and 0.1 M phosphate buffer (PB), pH 7.4, followed by 2% PFA (75 mL). Brains were removed and cut into 4–5 mm coronal slabs consisting of the entire rostrocaudal extent of the hippocampus and the EC and were then postfixed in 2% PFA for 24 hours and kept in 0.1 M PB, pH 7.4. Coronal sections of the brain were cut into 40–50 μm with vibrating microtome (VT1000S; Leica, Milton Keynes, UK). Free-floating sections in 0.1 M PB, pH 7.4, were collected and stored in cryoprotectant solution containing 25% sucrose and 3.5% glycerol in 0.05 M PB at pH 7.4. Coronal vibratome sections at levels −1.58/−2.46 mm (hippocampus) and −2.30/−3.88 mm (EC) posterior to Bregma were selected for immunohistochemistry according to the mouse brain atlas of Paxinos and Franklin (Paxinos and Franklin, 2004). Immunostaining of GFAP, GS, and s100β was performed using specific monoclonal mouse antisera against GFAP (anti-GFAP; Sigma-Aldrich, UK; #G3893) and GS (anti-GS; Millipore, UK; MAB302), respectively, and polyclonal rabbit antisera against s100β (anti-GS; DAKO; Z0311). Careful examination was performed on all brain sections for discarding eventual age-associated pathology, such as the presence and/or development of tumors; however, no experimental animal showed this condition in any of the different age groups.

GFAP- and s100β-positive astrocytes were imaged using confocal microscopy (Leica SP5 upright). Parallel confocal planes were superimposed, and morphological analysis was carried out by "Cell analyst" (Chvatal et al., 2007) using digital filters (average 3 × 3, convolution, gauss 5 × 5, despeckle, simple objects removal) to determine the surface, volume, and somata volume of GFAP/s100β-positive astrocytes in the dentate gyrus (DG) and cornu ammonis 1 (CA1) subfields of the hippocampus and in the entire EC. GS-positive astrocytes were imaged using light microscopy (Nikon Eclipse 80i microscope) coupled with an 8001 MicroFIRE camera. The cellular areas of GS-positive astrocytes were measured using Image I 1.32j (National Institutes of Health, USA).

Unpaired t test was used to examine the differences in surface, volume, and somata volume of GFAP-labeled cells at different age groups and the differences in surface area of GS-positive cells at 3 and 24 months. Data are expressed as mean ± standard error of the mean.

3. Results

3.1. General description of astroglial profiles identified with different markers

GFAP-positive astrocytes showed a characteristic stellate shape and multiple branched morphology (Fig. 1A–L), although there are clear differences between the hippocampus and the EC. Hippocampal astrocytes emanate several primary processes with numerous secondary processes, all of them extending radially (Fig. 1A–H). Astrocytic GFAP profiles in the EC show less branches with very little number of secondary processes (Fig. 1I–L). Similarly, distribution of astrocytes differed between the 2 brain regions. Astrocytes in the hippocampus uniformly cover the whole parenchyma with the pyramidal and granular cell layers being the only exception, where fewer astrocytes are present. In the EC, the presence of GFAP-positive astrocytes was less prominent (Olabarria et al., 2010; Yeh et al., 2011).

Cells immunoreactive to s100β (s100β-IR) showed typical astrocytic stellate shape in both the hippocampus and the EC with round somata and multiple branched processes (Fig. 3); proximal processes extend outward from the somata in radial manner, whereas the distal processes branched in random order. Both in the hippocampus and the EC, the s100β-IR cells were widely and evenly distributed, with the exception of granular and pyramidal cell layers of the DG and CA1 subregions of the hippocampal formation, where fewer s100β-IR are present.

GS-positive astroglial profiles displayed small round cell bodies with primary branches and few secondary processes extending randomly and radially (Fig. 5). In hippocampal subfields, DG and CA1, the distribution of GS-positive astrocytes was similar to GFAP- and s100β-positive astrocytes, being widely present in the whole regions despite fewer GS-positive astrocytes found in the pyramidal and granular cell layers as we previously described (Olabarria et al., 2011). In the EC, GS-positive astrocytes were evenly and extensively distributed throughout the entire region with only partial and/or minimal colocalization of GS-IR with GFAP labeling. Of note, most EC astrocytes were GFAP negative with 3 populations, accounting only 10.3% for GS/GFAP-IR and 11.3% for single positive GFAP-IR cells, whereas 78.1% of all astrocytes in EC were GS-IR single positive cells (Yeh et al., 2013).

3.2. Astrocytes in the DG show a progressive age-related increase in GFAP-IR profiles

GFAP-positive astroglial profiles in DG were similar at 3 and 9 months of age (Figs. 1A and B and 2A–C). At 18 months of age, we detected a significant increase in GFAP surface by 78.56% (1793.84 ± 138.53 vs. 1004.64 ± 174.88 μm^2, $p = 0.0228$), volume by 95.89% (665.95 ± 64.18 vs. 339.96 ± 70.83 μm^3, $p = 0.0225$), and somata volume by 128.14% (274.03 ± 25.90 vs. 120.11 ± 28.19 μm^3, $p = 0.0107$) compared with 3-month-old mice (Figs. 1A and C and 2A–C). The increment was also evident comparing with 9 months of age, the GFAP surface being increased by 83.41% (1793.84 ± 138.53 vs. 978.03 ± 165.58 μm^2, $p = 0.0178$), volume by 104.99% (665.95 ± 64.18 vs. 324.87 ± 68.97 μm^3, $p = 0.0184$), and cell body volume by 115.25% (274.03 ± 25.90 vs. 127.31 ± 31.32 μm^3, $p = 0.0222$) (Figs. 1B and C and