Delayed neurodegeneration and early astrogliosis after excitotoxicity to the aged brain

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Abstract

Excitotoxicity is well recognised as a mechanism underlying neuronal cell death in several brain injuries. To investigate age-dependent differences in neurodegeneration, edema formation and astrogliosis, intrastriatal N-methyl-D-aspartate injections were performed in young (3 months) and aged (22–24 months) male Wistar rats. Animals were sacrificed at different times between 12 h and 14 days post-lesion (DPL) and cryostat sections were processed for Toluidine blue, Fluoro-Jade B staining, NeuN and GFAP immunohistochemistry. Our results show that both size of tissue injury and edema were reduced in the old subjects only up to 1DPL, correlating with a slower progression of neurodegeneration with peak numbers of degenerating neurons at 3DPL in the aged, contrasting with maximum neurodegeneration at 1DPL in the young. However, old animals showed an earlier onset of astroglial response, seen at 1DPL, and a larger area of astrogliosis at all time-points studied, including a greater glial scar. In conclusion, after excitotoxic striatal damage, progression of neurodegeneration is delayed in the aged but the astroglial response is earlier and exacerbated. Our results emphasize the importance of using aged animals and several survival times for the study of acute age-related brain insults.

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1. Introduction

Normal brain aging is characterised by macroscopic changes including decreased brain weight and volume, and microscopic alterations such as changes in vasculature, blood–brain barrier permeability, modifications in the extracellular compartment, and both cellular and biochemical alterations in glia and neuronal cells (Scanhill et al., 2003; Shah and Mooradian, 1997). Glial cells show activated/reactive phenotypes with changes in cell morphology and metabolism and in some cases also an increase in cell numbers (Amenta et al., 1998; Peinado, 1998; Unger, 1998). Neuronal cells typically accumulate intracellular macromolecules like lipofuscin, which, together with the well known aging-induced increase in oxidative stress and an important decline in neurotransmission, induces a progressive loss of neuronal function (Finch, 2003; Finkel and Holbrook, 2000; Floyd and Hensley, 2002; Segovia et al., 2001). Undoubtedly, these changes influence the susceptibility and response of the aging brain to damage and may explain why aging is the major risk factor for higher incidence of brain injuries and worse outcome after an insult (Brown et al., 2003; Bruns and Hauser, 2003; Nakayama et al., 1994; Wang et al., 2003; Woo et al., 1992). However, it should be noted that most of the available experimental work use young animals to study acute CNS insults which are epidemiologically associated with aging, like brain ischemia or stroke (Bonita et al., 1997; Truelsen and Bonita, 2003).

A common mechanism underlying neuronal cell death in such acute injuries is excitotoxicity (Dirnagl et al., 1999; Doble, 1999; Mattson, 2003), a phenomenon that takes place when excessive activation of glutamate receptors leads to a series of intracellular processes inducing cell
death (Mattson, 2003; Olney, 1969). Of all glutamate receptors, N-methyl-D-aspartate (NMDA) receptors are considered the main leaders of these processes due to their high calcium permeability, as well as its induction of calcium mobilization from intracellular compartments (Mody and MacDonald, 1995). As such, overactivation of NMDA receptors by exogenous application of agonists has largely been used as a model of experimental brain injury in the postnatal (Acarin et al., 2001), adult (Dietrich et al., 1992; Kollegger et al., 1993; Stewart et al., 1986) and also the aged brain (Suzuki et al., 2003).

Moreover, it is now well established that besides primary neuronal cell death, inflammatory, toxic and protective molecules produced by surrounding activated glial cells determine the final outcome of brain damage. Reactive astrocytes play many important roles, both detrimental and beneficial for neuron survival and are the main components of the glial scar (Anderson et al., 2003; Nedergaard and Dirnagl, 2005; Pekny and Nilsson, 2005), which provides structural support to injured tissue, but at the same time it is extremely inhibitory for axonal regeneration (Bovolenta et al., 1993). Nevertheless, besides these general features of astrogliosis, the intensity and temporal pattern of the astroglial response observed after brain injury can vary depending on the type of stimulus, the CNS region and age of the animals (Acarin et al., 1999; Raivich et al., 1999; Stoll et al., 1998). In the normal aging brain, up to date, the most outstanding and characteristic change described in astrocytes is cell hypertrophy associated with a significant increase in the expression of the intermediate filament protein glial fibrillary acidic protein (GFAP) (Bronson et al., 1993; Finch, 2003; Landfield et al., 1977), changes that are commonly found in reactive gliosis in the adult brain (Eng et al., 2000) and that will obviously influence the astroglial response in the lesioned aging brain. Despite this fact, again most of the studies on the astroglial response are based on the use of young adult animals and little and contradictory data is available on the extent and the temporal profile of reactive gliosis in the lesioned aging brain.

In this context, based on the hypothesis that neurodegeneration and the glial response may differ after damage to the aged CNS, in comparison to the young adult brain, the aim of the present study was to investigate, after an excitotoxic lesion, the age-dependent alterations in the extent and the temporal profile of neurodegeneration, brain edema and astroglial reactivity, three important parameters that determine final lesion outcome. For this purpose, brain damage response was followed from 12 h until 14 days post-lesion in young and aged rats.

2. Materials and methods

2.1. Study population

Experimental animal work was conducted using male Wistar rats of two different age groups: 46 young animals (3- to 4-month-old) and 51 old animals (22- to 24-month-old) were used. The subjects were distributed in three groups which will be referred as: intact controls, saline-injected controls and NMDA-injected lesioned animals. Animals were housed individually in cages with free food and water supply, in an environment with controlled temperature (22 °C), humidity (55%) and light/dark cycle (12 h). All experimental work was conducted according to established European Union bioethic directives and was approved by the ethical commission of the Universitat Autònoma de Barcelona. During all the process unnecessary animal suffering was avoided.

2.2. Excitotoxic lesions

Under isofluorane gas anesthesia, rats were placed in a Kopf stereotaxic frame. A hole in the skull was opened with a drill, and 120 nmol of NMDA (Sigma, M-3262, St. Louis, USA), diluted in 1 μl of saline solution (0.9% NaCl) were injected into the striatum (caudate–putamen, coordinates A = +0.12, L = −0.3; V = −0.45 cm) of the right hemisphere, with a Hamilton microsyringe coupled to an automatic microinjector (Stoelting, Illinois, USA) at a speed of 0.2 μl/min. After the injection, the needle was kept 10 min in order to facilitate diffusion into the striatum and minimize reflux. Finally, operated animals were maintained at normothermia by means of a thermal pad inside an incubator until they recuperated from anesthesia. Saline-injected controls followed the same procedure but were injected with 1 μl of saline solution. The groups of intact control animals did not receive any injection.

2.3. Sacrifice and histologic processing

After the survival times of 12 h post-lesion (HPL) 1 day, 3 days, 5 days, 7 days, and 14 days post-lesion (DPL), animals were anaesthetised and intracardially perfused for 10 min with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). At least 5 NMDA-injected lesioned animals and 2 saline controls per age and survival time were sacrificed, in addition to 4 intact control adult animals and 8 intact control aged animals. After perfusion, brains were quickly removed, postfixed for 4 h at 4 °C in the same fixative and immersed for cryoprotection in a 30% sacarose solution in 0.1 M phosphate buffer (pH 7.4) until freezing. For each animal, 15 series of 30 μm thick parallel frozen coronal sections (thus, each section 450 μm apart from adjacent ones) were obtained with a Leitz cryostat, and either stored free-floating in antifreeze solution at −20 °C or mounted on gelatin-coated slides and stained with toluidine blue. After washing, slides were dehydrated in alcohol, cleared in xylene, and coverslipped with DPX. These slides were used for the histologic control of the intracerebral injection, the study of the size of injury and ipsilateral hemispheric swelling.
2.4. Fluoro-Jade B staining

For the study of degenerating neurons, sections were stained with Fluoro-Jade B as described (Schmued and Hopkins, 2000). Briefly, free-floating sections were mounted and air dried overnight. After dehydration and rehydration in graded ethanol, sections were rinsed in water and oxidized with 0.06% potassium permanganate (MnO$_4^-$K in water) for 15 min. Next, sections were rinsed with distilled water and immersed in 0.0004% Fluoro-Jade B (1FJ-B, Histo-Chem Inc., Jefferson USA) + 1% glacial acetic acid for 20 min. After rinsing, slides were air dried, cleared in xylene and coverslipped in DPX.

2.5. Immunohistochemistry

For the visualization of astrocytes, parallel free floating sections were processed for the immunohistochemical demonstration of GFAP and for neuronal labelling sections were immunohistochemically processed for the Neuronal nuclear antigen (NeuN). In both cases, sections were washed in tris buffered saline (TBS) 0.05 M, pH 7.4, and after endogenous peroxidase blocking (10 min with 2% H$_2$O$_2$ in 70% methanol), sections were washed in TBS, in TBS + 1% Triton X-100 and treated with blocking buffer (BB) (10% foetal calf serum in TBS + 1% Triton X-100) for 30 min. Afterwards, sections were incubated overnight at 4 °C and 1 h at room temperature, with either a polyclonal rabbit anti-cow GFAP antibody (DakoMats Z-0334, Dako A/S, Glostrup, Denmark) (1:1800 in BB); or a monoclonal mouse anti-NeuN (Chemicon International, MAB 377, Temecula, CA, USA) (1:1000 in BB). After the incubation, sections were rinsed in TBS + 1% Triton X-100 and treated with blocking buffer (BB) (10% foetal calf serum in TBS + 1% Triton X-100) for 30 min. Afterwards, sections were incubated overnight at 4 °C and 1 h at room temperature, with either a polyclonal rabbit anti-cow GFAP antibody (DakoPatts Z-0334, Dako A/S, Glostrup, Denmark) (1:1800 in BB); or a monoclonal mouse anti-NeuN (Chemicon International, MAB 377, Temecula, CA, USA) (1:1000 in BB). After the incubation, sections were rinsed in TBS + 1% Triton X-100 and incubated for one hour at room temperature with either biotinylated secondary antibodies: anti-rabbit Ig (Amersham Biosciences RPN1004V1, UK) (diluted 1:200 in BB); or anti-mouse Ig (Amersham Biosciences RPN1004V1, UK) (diluted 1:200 in BB). Afterwards, sections were rinsed again and incubated for 1 h at room temperature with avidin-peroxidase (DakoPatts P0364, Dako A/S, Denmark) diluted 1:400 in BB and washed in TBS and in tris buffer (TB). Peroxidase reaction product was visualized with 100 ml of TB containing 50 mg of 3,3'-diaminobenzidine (DAB) and 33 μl of hydrogen peroxide for 4-5 min. Finally sections were rinsed in TB, mounted on gelatine-coated slides, dehydrated, cleared in xylene, and coverslipped in DPX. As negative controls the primary antibodies were omitted.

2.6. Quantification: general considerations

Sections from intact controls, NMDA injected and saline-injected control animals were studied through a Nikon Eclipse E 600 microscope interfaced to a DMX 1200 camera and a PC. Pictures of representative areas were taken at different magnifications using the software ACT-1 2.20 (Nikon Corporation). A minimum of 4 NMDA-injected animals for each survival time and age group were used. Researchers were blinded to sample identity. For each animal and staining procedure, 4 sections in the injury core comprised between 1 mm/–0.3 mm from bregma were analysed. Anatomical landmarks (aspect, size and position of the anterior commissures, corpus callosum, septum, lateral ventricles, striatum, nucleus accumbens) were used to ensure that parameters were analysed at similar levels within and between groups.

2.6.1. Quantification of neurodegenerative area, brain edema and astroglial reactivity

Selected brain sections were scanned with a Nikon LS-1000 assembled to a Macintosh computer. National Institute of Health Image software (N.I.H. 1.62) was used to obtain the following area values in mm$^2$: (i) contralateral hemisphere (CLH); (ii) contralateral ventricle (CLV); (iii) corrected contralateral hemisphere (cCLH) obtained as CLH-CLV; (iv) ipsilateral hemisphere (ILH); (v) ipsilateral ventricle (ILV); (vi) corrected ipsilateral hemisphere (cILH) obtained as ILH-ILV; (vii) area of tissue injury in the ipsilateral hemisphere, identified in toluidine blue processed sections in basis of its decreased staining; and (viii) area of astroglial reactivity in the ipsilateral hemisphere, measured in sections processed for GFAP, as the area showing higher staining than the contralateral hemisphere of the same section.

In order to avoid misinterpretation of data due to the significantly smaller hemisphere size in the old control subjects (see Section 3), results are shown as percentage of total area in the ipsilateral hemisphere: (i) % of tissue degeneration = (area of tissue degeneration/cILH) × 100; and (ii) % of astroglial reactivity = (area of astroglial reactivity/cILH) × 100. As previously described (Fujimura et al., 2001), brain edema was calculated indirectly from values of the ipsilateral hemisphere as follows: % of hemispheric swelling (brain edema) = (cILH/cCLH) × 100. Accordingly, positive results were considered indicators of brain edema, whereas negative results would point to hemispheric collapse or atrophy.

2.6.2. Quantitative study of neurodegeneration

In order to avoid misinterpretation of data due to possible reduction in striatal neuronal cell numbers with aging (see Section 3), NeuN+ profiles were assessed in control aged and young striatum. Additionally, to address the percentage of remaining neurons, NeuN+ profiles were assessed in the 14DPL group. Fluoro-Jade B+ profiles were assessed in all control and injured animals. Region of interest (striatum) was delimited by the lateral ventricle medially, the corpus callosum dorsally and laterally, and by a line drawn between the two anterior commissures ventrally. For each section, 4 pictures were taken at 10×, comprising the whole striatal area, and all profiles clearly identified as specifically labeled neurons were manually counted. The number of Fluoro-Jade B+ neurodegenerating profiles and NeuN+ remaining profiles were expressed...
in relation to age-matched control striatal NeuN+ neuronal profiles as follows: (i) percentage of degenerating neurons = (FJ-B+ profiles/NeuN+ profiles in age-matched control animals) × 100; (ii) percentage of remaining neurons = (NeuN+ profiles 14DPL/NeuN+ profiles in age-matched control animals) × 100.

2.7. Statistical analysis

Statistical analysis was performed with SPSS 11.01. Striatal neuronal cell numbers, hemispheric area and ventricle size in control animals were studied by means of one-way ANOVA followed by Scheffe post hoc comparison. Tissue injury, neurodegeneration, edema and astroglial reactivity percentage results were studied by means of two-way ANOVA. All results are presented as mean values ± SEM, percentage results were studied by two-way ANOVA. Overall, in aged animals, evolution of the degenerating area was more regular, varying from 15.5 ± 3.6% to 7.50 ± 1.2% of ipsilateral hemisphere, whereas in the young it changed from 27.7 ± 4.5% at 1DPL to 2.6 ± 0.6% of ipsilateral hemisphere at the last time studied.

Brain edema, shown as the percentage of ipsilateral hemispheric swelling, was marginally significantly bigger in young subjects only at 1DPL (Fig. 2C). As observed with injury size, no differences between age groups were found at later time points, although differences between survival times were observed at both ages. Whereas in young subjects brain edema increased between 12HPL and 1DPL and diminished between 3 and 5DPL, this pattern was delayed in the aged animals, where brain edema slightly increased between 1 and 3DPL and hemispheric swelling was reduced between 5 and 7DPL (Fig. 2C). After 7DPL brain edema was not observed at any age group. Instead, in the aged group a tendency to hemisphere collapse was observed, giving negative values both at 7 and 14DPL.

The temporal analysis of neurodegeneration within the lesioned striatum was carried out by Fluoro-Jade staining, a marker for degenerating neurons. Fluoro-Jade B+ was observed in the soma of cells with the morphology of striatal neurons, in both young and aged rats and at all time points studied (Fig. 3). Although Fluoro-Jade B+ labeling was observed in the whole striatal injured area at both ages, the higher number of stained cells as well as the strongest staining intensity were always found at the lesion core, especially in aged animals. Towards the periphery of the lesion, cellular labelling decreased in amount and intensity. The quantitative analysis of Fluoro-Jade B+ staining showed that the temporal dynamics of neurodegeneration were different between young and aged animals (Fig. 3I). Although young rats presented a slightly higher percentage of degenerating neurons than the aged at 12HPL, greatest differences were seen in the time of maximum neurodegeneration. Whereas young rats reached a peak percentage of Fluoro-Jade B+ labeling by 1DPL, in the aged, neurodegeneration developed to a maximum percentage of degenerating neurons at 3DPL. At 5DPL both ages showed similar percentages of neurodegeneration that were not different from 12HPL. It should be noted that aged animals showed Fluoro-Jade B+ labelling in white matter bundles at 7 and 14DPL, hindering a reliable counting at the latest time points. Finally, no changes in the percentage of...
remaining striatal NeuN+ profiles were seen between the young and the aged groups at 14DPL (Fig. 3J), the last time studied.

### 3.3. Astroglial reactivity

Aged and young animals injected with saline solution did not show differences in the pattern of astroglial response, both groups presented hypertrophied astrocytes only adjacent to the needle track (data not shown). In contrast, in animals injected with NMDA, the quantitative analysis of the area occupied by reactive astrocytes showed significant differences between ages and post-lesion times. The astroglial response developed earlier and was significantly greater in the aged subjects at all time points studied (Fig. 4). At both ages there was a significant increase in the area of reactive astrocytes between 1 and 3DPL and the maximum area was achieved at 3DPL, when 32.9 ± 6.1% of hemispheric area in the young and 58 ± 6.1% in the aged were occupied by reactive astrocytes. Longer survival times were characterised by a progressive reduction until 7DPL but no further reduction was detected at 14DPL (Fig. 4B), when the area occupied by reactive astrocytes was still significantly bigger in the aged subjects, implying the formation of a larger glial scar.

At 12HPL, the lesion core of both young and aged animals presented a punctuated background of GFAP immunoreactivity, but only in the aged animals scattered GFAP+ astrocytes were clearly distinguished (Fig. 5A–D). At 1DPL, whereas in the young animals the lesion core was still devoid of astrocytes, in the aged, signs of astroglial hypertrophy were perceived (Fig. 5E–H): In the cortex, GFAP overexpressing cells were observed, and in the striatum thick immunoreactive cellular extensions were mainly
detected. Interestingly, at 3DPL, the central core of the cortical or striatal lesion and in both young and aged rats, was devoid of GFAP+ cells (and filled mainly with microglia/macrophages, data not shown) and surrounded by reactive astrocytes (Fig. 5 I and J). In aged animals, these astrocytes were more strongly hypertrophied, especially striatal astrocytes that showed the thickest and shortest extensions (Fig. 5K and L). At 5DPL, cellular hypertrophy was increased and aged reactive astrocytes were still more hypertrophied than in the young brains (Fig. 5M–P). At 7DPL and in both age groups, the diminution in the area occupied by reactive astroglial cells (Fig. 4A) coincided with a concentration of reactive astrocytes in the lesion core. At this survival time, the grade of astroglial reactivity was similar between ages, and higher than the observed at earlier time-points (Fig. 5Q–T). At both ages, cortical astrocytes were more numerous, had thinner cellular processes and showed a different pattern of hypertrophy than striatal astrocytes where GFAP immunoreactivity concentrated in the soma, and cells displayed thicker and shorter extensions, especially in aged animals (Fig. 5T). At 14DPL in both ages, the presence of the glial scar was clearly observed, with more strongly packed and more hypertrophied astrocytes than at 7DPL (Fig. 5U–X). At this last time-point, the presence of vacuoles in the astrocytic soma was clearly evident (Fig. 5Y) although they were already observed for the first time at 3DPL in both age groups.

4. Discussion

4.1. Neurodegeneration and edema in the aged brain

By analysing the evolution of lesion size and neurodegeneration after an NMDA-induced excitotoxic lesion in
the striatum of young and aged rats we here describe, in comparison to the young rats, a decreased lesion size in aged rats until 1DPL and a concomitant slower progression of neuronal degeneration in the injury core. Interestingly, available studies using several experimental models of brain ischemia in aged rats show a remarkable variability in the results reported. Studies have either shown bigger injury size in the young brain (Shapira et al., 2002), no differences in the injury size between age groups (Badan et al., 2003; Brown et al., 2003; Duverger and MacKenzie, 1988; Fotheringham et al., 2000; Wang et al., 2003) or a bigger injury size in the aged brain (Davis et al., 1995; Futrell et al., 1991; Kharlamov et al., 2000; Sutherland et al., 1996). The only previous study which has used NMDA to induce excitotoxic damage in old (24 months old) and young adult (8 weeks) (Suzuki et al., 2003) showed no significant differences in injury size at day 1, but they found a trend towards more damage in the young (p = 0.16), the only time-point analysed. The several times lower dosage of NMDA used, the strain, and the site of NMDA injection could account for the lack of significance in their results.

It is not surprising that the variability of neuropathological findings becomes especially highlighted in studies based on aged subjects, where it sums to the intrinsic variability of aging. We here describe a significant reduction in the aged brains’ hemispheric area and in the number of striatal neuronal profiles, as it has been previously described (McNeill and Koek, 1990; Scahill et al., 2003; Stemmelin et al., 2000) and paralleling the presence of scattered Fluoro-Jade B+ degenerating neurons in the normal aged striatum. These differences stand out the need of presenting results as percentages of age-matched controls, when comparing aged versus young brains. In this regard, many existing work obviate the decrease in brain size (Fotheringham et al., 2000; Kharlamov et al., 2000; Suzuki et al., 2003; Wang et al., 2003), which could lead to misleading interpretations, as an equal injury size means a bigger percentage of lesion in a smaller brain. In this sense, variations in the lesion model, experimental procedure, specie and strain, age, post-lesion time analysed and the way of presenting results are particularly important when comparing studies on the young and aged brain’s response to an insult.
It is now well established that the final lesion outcome depends both on the injury size and the extent of neuronal cell death within the injured area. By using Fluoro-Jade B, a marker for degenerating neurons (Schmued and Hopkins, 2000), we have shown that whereas neurodegeneration is more rapid in the adult, showing maximum numbers of dying neurons at 1DPL, in the aged striatum the neurodegenerative process is slower and peaks at 3DPL. The delay in the neurodegenerative process may be probably explained by intrinsic factors in neuronal cells occurring during aging. In agreement, the most significant and consistent finding in glutamatergic transmission alterations occurring with aging is a decrease in NMDA receptor density which induces a previously reported diminution in the response mediated by this receptor (Segovia et al., 2001; Yamada and Nabeshima, 1998). Moreover, the lower metabolic rate, increased oxidative stress, inflammation and other biochemical CNS changes associated with aging (Finkel and Holbrook, 2000; Floyd and Hensley, 2002; Lee et al., 2000; Moeller et al., 1996), are stimulus reported to induce moderate tolerance against ischemia-induced neuronal death in the adult brain, a phenomenon known as ischemic tolerance or preconditioning (Dirnagl et al., 2003; Trendelenburg and Dirnagl, 2005). As examples, increased basal levels of nitric oxide, heat shock proteins (HSPs) and Nuclear Factor kappa B (NFκB) found in aged (Calabrese et al., 2004; Korhonen et al., 1997; Toliver-Kinsky et al., 1997), have all been related to an increased resistance to ischemic damage (Dirnagl et al., 2003) and could explain why the aging brain may respond better to the insult at first. However, it is well established that aged subjects present poor final lesion outcome attributed to a decreased capacity to adapt to new situations, which some authors have related to a slower and reduced over-induction of protective genes like HSPs and NFκB (Nakayama et al., 1994; Shamovsky and Gershon, 2004; Verbeke et al., 2001), and that could explain the delayed neurodegeneration we observe. However, it should be mentioned that in our model, the global percentage of remaining striatal neuronal profiles was not different between ages, suggesting that both the decreased amount of striatal neurons in control aged striatum as well as the impairment of neurochemical and neurophysiological parameters of remaining neurons may contribute to a worse final outcome characteristic of aged subjects. In this sense, several studies have demonstrated that aging is associated with a reduction in striatal dopamine as well as trophic activity (Ling et al., 2000) and that following neurotoxic treatment to the aged striatum, the regenerative-associated increase in the expression of neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) is highly diminished (Collier et al., 2005; Yurek and Fletcher-Turner, 2000, 2001), pointing to the idea that the aged striatum loses the ability to biochemically and trophically compensate for neural damage. Finally, slower neurodegeneration observed in aged brains, may also be related to the delayed marginal increase in edema formation, as brain edema causing swelling of the affected hemisphere is considered an important component of the neuropathological process (Kimelberg, 2004; Lin et al., 1993; Wang et al., 2003). Formation of edema...
depends on blood–brain barrier breakdown and the movement of liquid from the extracellular to the intracellular compartment, producing secondary injury by alteration of brain homeostasis and mechanic pressure (Kimelberg, 2004). Although brain edema has been sparsely studied in aged animals, it is clear that many factors implicated in

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Fig. 5. Astroglial reactivity after excitotoxic damage to the young adult and aged brain from 12HPL to 14DPL. Only in the aged animals GFAP+ astrocytes are observed within the first 1DPL (A–H): in the cortex, GFAP+ cells are clearly observed (B,F), while in the striatum immunoreactive thick cellular extensions are mainly detected (D,H). At 3DPL, in the cortex, a core free of GFAP+ cells (asterisks in I and J) surrounded by reactive astrocytes is seen at both ages (I,J), although astrocytes are more reactive in the aged (I, K in comparison to I, K, respectively). At 5DPL, cellular hypertrophy is increased (M–P) and glial scar is observed at 7DPL and 14DPL at both ages (Q–X), although greater cell hypertrophy is clearly seen in the aged. The presence of vaquoles in the astrocytic soma is clearly evident in astrocytic soma at 14DPL (arrow in Y). The morphological differences between cortical and striatal astrocytes are maintained. Scale bars (A–Y) = 50 μm.
et al., 1998), are elevated in the aged rodent (Bodles and
lators of astroglial reactivity (Raivich et al., 1999; Stoll
necrosis factor alpha and interleukin-6, well known modu-
lar calcium (Finkbeiner, 1992; Raivich et al., 1999). In
tion in distal sites depends on the propagation of intracel-
many in rats, where neuronal death is limited. In contrast,
available results on experimental models where massive
atal age-induced astroglial activation (Scheff et al., 1980),
or because aged astrocytes are less sensitive to signalling
be our model, are con-
duced by surrounding activated glial cells and infiltrating
leukocytes determine the final outcome of brain damage.
Among other mechanisms, oxidative stress and inflamma-
tion mediated by proinflammatory cytokines and enzymes
(Iadecola, 1997; Minghetti and Levi, 1998) are considered
to be the leading factors contributing to delayed cell death
(Dirnagl et al., 1999).
In conclusion, these results emphasize the importance of
using aged animals for experimental models of insults relat-
ed to aging, and stands out the need of taking into account
the changes related to normal aging in addition of multiple
parameters along different survival times to evaluate dam-
age progression in the aged. Noteworthy, the delay in the
neurodegenerative process may imply a longer therapeutic
window in the aged.

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