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Enriched environmental conditions reverse age-dependent gliosis and losses of neurofilaments and extracellular matrix components but do not alter lipofuscin accumulation in the hindlimb area of the aging rat brain

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Abstract

We provide a description of a correlation of lipofuscin accumulation and expression of glial fibrillary acidic protein in the cerebral cortex of aged rats. Glial fibrillary acidic protein showed a complementary distribution pattern to perineuronal nets, visualized with *Wisteria floribunda* agglutinin. With progressing age (12–36 months), a strong increase of lipofuscin and gliosis occurred in functionally characterized cortical areas, whereas a concomitant, area-specific loss of perineuronal nets was found in the cortical somatosensory representation of the hindlimbs. In contrast to lipofuscin accumulation and increased gliosis, the loss of perineuronal nets and the reduction of non-phosphorylated neurofilament H were in part reduced or prevented by housing the animals under enriched environmental conditions between 33 and 36 months of age. Especially the reduction of astrocytosis by 20% which coincided with a reduction in the loss of extracellular matrix components involved in forming the glia–neuron-interface demonstrates, that the aging cortex retains its potential for functional plasticity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Reactive astrocytes; Lectin-binding sites; SMI 32; Motor cortex; Somatosensory cortex

1. Introduction

On a behavioral level, old rats are known to show a number of age-related changes (O'Hare et al., 1999; Pelosi and Blumhardt, 1999), such as a characteristic impairment of the sensorimotor system, which is most strikingly expressed in a walking impairment involving the hindlimbs (Schuurman et al., 1987; Ingram, 1988; Stoll et al., 1996). Our studies (Spengler et al., 1995) also revealed profound age-related electrophysiological alterations of the cortical hindpaw representation of aged rats reflected by a severalfold enlargement of their receptive field representations. When such old rats became transferred to an enriched environment, receptive fields of neurons in the cortical hindpaw representation area decreased again to nearly the size found in younger adults. Therefore, the question arises as to whether these electrophysiological and functional findings would be accompanied by intracortical, morphological alterations. In addition, it is important to clarify whether these changes are caused by more general, age-related degenerative processes, or whether they truly are use-dependent alterations.

We postulate, that some aspects of age-related changes reflect plastic reorganization as a consequence

Abbreviations: FL, forelimb, forepaw; GFAP, glial fibrillary acidic protein; HL, hindlimb, hindpaw; MC, motor cortex; SC, somatosensory cortex; WFA, *Wisteria floribunda* agglutinin; S.D., standard deviation.

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of prolonged disuse of the hindlimbs rather than a process of degeneration. This hypothesis is based on the fact that in 3-year-old rats the sensorimotor behavior of the forepaw remains largely unaffected. If solely degeneration were responsible for age-related alterations, one would expect similar changes to occur in the fore- and the hindpaw representation areas. Electrophysiological analysis of the area of forepaw representation in a previous study using exactly the same experimental setup and rat strain, revealed, however, no age-related alterations of the receptive fields or the respective cortical representational maps (Berkefeld et al., 1996). We have expanded this model by introducing anatomical techniques to investigate the possible cytoarchitectonic and anatomical basis for the electrophysiologically observed functional changes.

Nerve cells within the cortex of man are known to accumulate lipofuscin during their life span. The amounts and the characteristic patterns of that pigment distribution may serve as criteria for staging of age-induced alterations in the human brain (Braak, 1979, 1983). Only very few animal models for pigment accumulation in the brain are described (Braak et al., 1984; Koppang, 1973/4; Purpura and Baker, 1978). Besides the pattern of lipofuscin accumulation, the distribution of reactive glia and the glial–neuron-interface (Celio et al., 1998) were analyzed. Glial fibrillary acidic protein (GFAP) has been shown to be expressed by reactive astrocytes (Bignami and Dahl, 1976; Eng and Dearmond, 1983; Eng et al., 1987; Eng, 1988). GFAP positive astrocytes are responsible for an age-related, chronically developing gliosis (Murphy, 1993). In normal tissue, glial cells are involved in establishing a more specialized glial–neuron-interface in the direct environment of synaptic contacts. This glial–neuron-interface which is part of the extracellular matrix (Celio et al., 1998), can be visualized by means of lectin binding (Naegele and Katz, 1990; Härtig et al., 1992, 1999; Brückner et al., 1994; Seeger et al., 1996). Similar to previous reports, *Wisteria floribunda*-agglutinin (WFA) was used to study age-related alterations of this matrix component. Among others, the interaction between glia and neuron by means of extracellular matrix is important for the maintenance of the acid–base steady-state and is subject to modification by pH and microenvironmental changes (Chesler, 1990; Härtig et al., 1999). In addition, and in order to also focus on neurons, neurofilamental alterations were studied. Neurofilaments and their state of phosphorylation have been identified as sensitive markers of pathology related as well as during regeneration processes (Wong et al., 1995). Furthermore, transgenic mouse models in which the heavy or light subunits of neurofilament protein are overexpressed, display a motor neuron disease similar to amyotrophic lateral sclerosis. Therefore, cytoskeletal alterations (both hypo- and hyperphosphorylations) were described as being a defining attribute of the degenerative processes (Morrison and Hof, 1997). In Alzheimer's disease neurofibrillary tangles occur primarily in neurofilament-protein-rich neurons by aggregation of cytoskeletal elements into tangles, indicating that alterations within neurofilaments may be good candidates to detect age-related changes at the neuronal level.

2. Material and methods

2.1. Experimental animals

We used 45 hybrid Fischer 344* Brown Norway rats (FBNF1 rats) as described by Van der Staay and Blokland (1996) of following ages: 3 months (three animals as controls), 11–12 months (nine animals), 24 months (nine animals), 36 months (16 animals divided into eight animals housed in standard environment and and eight animals housed under enriched living conditions during the last 3 months of their life). Groups of 4–5 control rats were kept under standard conditions in littered standard Type IV cages ($54 \times 33 \times 18.5$ cm^3, length × width × height). The dimensions of the cages containing the 'enriched environment' were $100 \times 60 \times 80$ cm^3 ($l \times w \times h$). The interior was changed weekly and consisted of large irregular-shaped styrofoam blocks of variable size, cardboard boxes and several wooden ladders. Food-pellets and sunflower seed were supplied at variable locations to reinforce extensive explorative behavior. Behavior and electrophysiological investigations have been described by Berkefeld et al. (1996), Godde et al. (1995, 1996, 2000).

2.2. Preparation of sections

Under ether anaesthesia, the animals were transcardially perfused with physiological saline, followed by about 200 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and post-fixed for 2 h in the same fixative.

Brains were cryoprotected by immersion in phosphate buffer containing 15% sucrose for 1 day, followed by 30% sucrose overnight at +4 °C. Serial 30 μm frozen sections were used for visualization of lipofuscin and for immunohistology. The cross sectional distance between the sections used for the immunofluorescence technique was 90 μm, and for the streptavidin peroxidase technique, the cross sectional distance was150 μm. For lipofuscin visualization by autofluorescence, the sections were mounted on slides, dried and coverslipped with Entellan (Merck, Darmstadt). The pattern of lipofuscin could be directly detected under UV light. For light microscopical staining, neighboring sections were treated with 0.3% H_2O_2 in 0.1 M phosphate buffered

saline (pH 7.4) for 30 min. After several rinses with 0.1 M phosphate buffered saline (pH 7.4) they were incubated with normal serum according to the secondary antibodies used.

2.3. Lectin histochemistry

After treatment with 5% bovine serum albumin for 1 h, the sections were incubated with biotinylated WFA (b-WFA, Sigma L-1766, Munich) at a concentration of 10 μg b-WFA/ml 0.1 M Tris buffered saline (pH 7.4) containing 2% bovine serum albumin overnight at +4 °C. After several rinses the sections were treated with the streptavidin-peroxidase solution (Sigma-Immunochemicals) and the reaction product was visualized by diaminobenzidine/H_2O_2 (Sigma).

2.4. Immunohistochemistry

After treatment with 10% goat normal serum overnight, further sections were incubated with the primary antibody against GFAP (monoclonal, DAKO) at a dilution of 1:100 and against SMI 32 (Biotrend, Cologne, Germany) at a dilution of 1:1000, respectively. After several rinses the secondary antibody, biotinylated goat-*anti*-mouse, was diluted 1:50 (Vector), followed by the incubation in streptavidin peroxidase (Sigma-Immunochemicals) for 1 h. Visualization of the reaction product was performed with diaminobenzidine/H_2O_2. Sections were rinsed with Tris buffer, mounted on glass slides, air-dried and coverslipped with Entellan (Merck, Heidelberg).

For colocalization studies, we first used b-WFA (see above), which was visualized by incubating sections for 4 h in phosphate buffered saline (pH 7.4) containing Cy2-conjugated streptavidin (Amersham, diluted 1:50). Sections were rinsed and placed in Tris buffered saline (pH 7.4) containing a Cy3-labeled monoclonal antibody against GFAP (Sigma) at a dilution of 1:400 (+4 °C, 12 h). They were then rinsed and coverslipped. Control sections were treated with non-specific mouse antibodies (IgG1, DAKO) diluted and applied similarly to the specific antibodies. Sections were studied light or fluorescence microscopically with a photomicroscope 'Axiophot' (Zeiss, Germany) equipped with epifluorescence. Double labeled sections were examined in the LSM 410 confocal laser-scanning microcope (Zeiss, Germany) with excitation wavelengths at 488 and 568 nm.

2.5. Measurements of grey values

The interactive measurements of grey values were carried out using the software of the Kontron–Videoplan-System (Kontron, Zeiss, Germany) and the 'frozen image tool'. For each animal and each time point we analyzed three subsequent sections. In each section stained for light microscopy or native (with lipofuscin autofluorescence) section we scanned six areas, which were placed within each hemisphere into somatosensory representation areas for the forelimb (three areas) and the hindlimb (three areas) according to Zilles (1985). These areas of interest were further divided in accordance to the cortical layers in an supragranular area including cortical layers I–IV and an infragranular area including layers V and VI. The scanned area was restricted to L I–IV or to L V–VI, respectively. The system software of the Videoplan provided the mean grey value (average ± S.D.) and the medians for each experimental group of animals. The *U*-test was used to compare the data sets of the different files and to analyze the level of significance for the differences among the various age groups. Since highest lipfuscin levels were detected as the lowest grey value (due to autofluorescence visualization), and high amounts of GFAP correspond to high grey values, the absolute values were expressed as percent. In the case of lipofuscin autofluorescence the lowest averaged grey value was set at 100%, whereas for GFAP immunoreactivity the highest average of measured grey value was set at 100%.

3. Results

3.1. Patterns of lipofuscin accumulation and GFAP expression

An age-dependent accumulation of lipofuscin and GFAP expression were one of the most evident signs of aging. The first deposits of dusty lipofuscin granules in the rat brain occurred at the age of about 1 year in most cortical regions. Small amounts of lipofuscin were co-distributed with a few GFAP expressing astrocytes. The pattern of lipofuscin distribution was more homogeneous than has been described for the aged human brain (Braak, 1983), and did not allow the establishment of a pigment-based architectonic map as known from humans. With progressing age (2 years), enlarged lipofuscin granules accumulated in the somata of many neurons. The marked distribution of lipofuscin in 3-year-old rats was, however, more concentrated in neurons of the primary motor cortex (MC) and the somatosensory cortex, with a slightly higher, selective accumulation in HL (Figs. 1 and 2), whereas neurons within all other cortical areas contained still the small, dust-like lipofuscin granules known from younger animals. The largest clusters of lipofuscin granules were present in the large pyramidal shaped cells of L V of the MC. The areal pattern for the neuronal distribution of lipofuscin corresponded well with the pattern found for the distribution of GFAP-expressing, reactive astro-

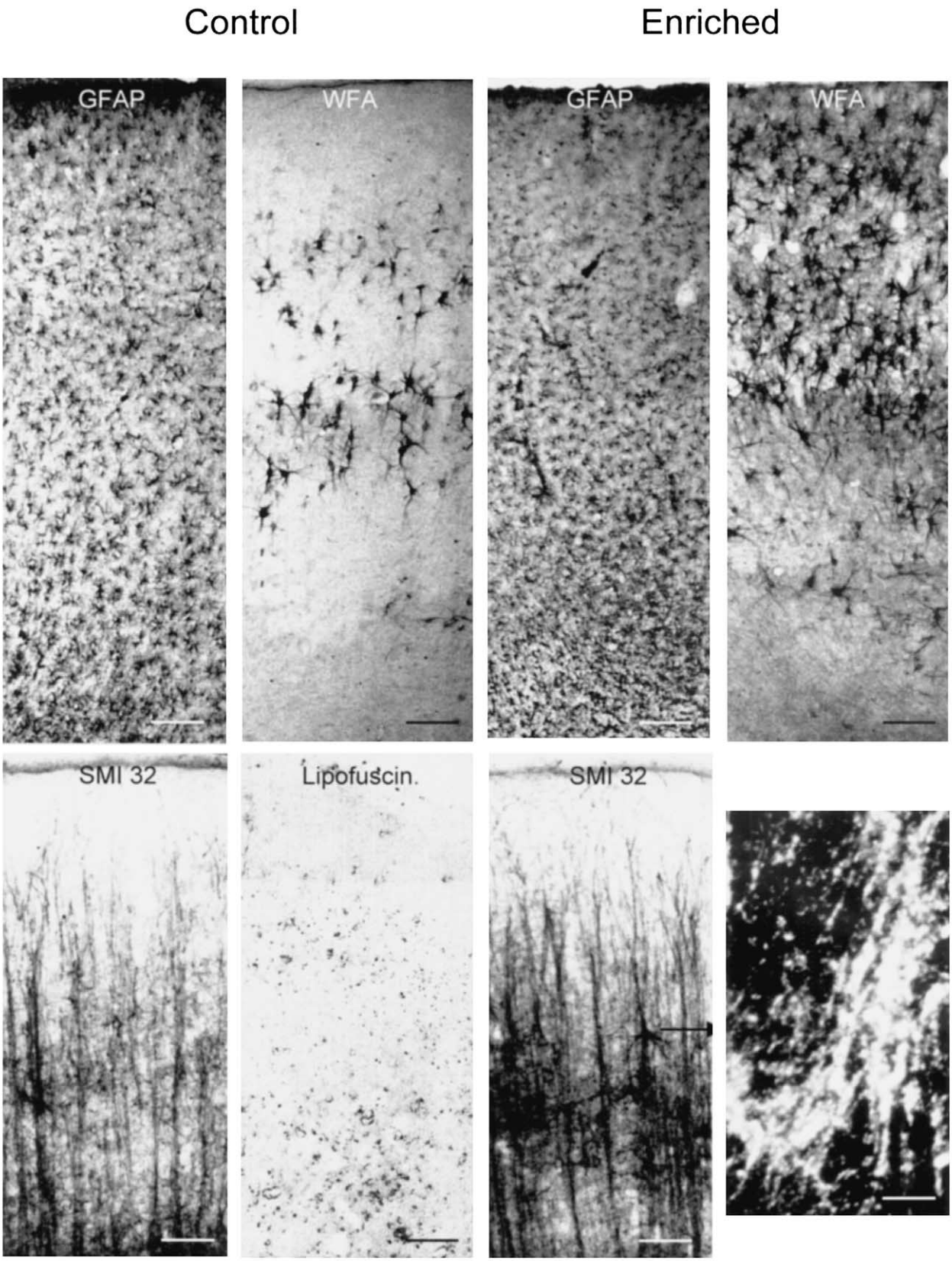

Fig. 1. Comparison of GFAP, non-phosphorylated neurofilament SMI 32 expression and WFA binding within HL at the age of 3 years in rats housed under standard conditions (Control) and those which had spent the last 3 months under enriched conditions (Enriched): Whereas the reduction in GFAP immunoreactivity is moderate a clear increase of WFA binding and SMI 32 expression within the supragranular cortical layers of HL becomes evident. Laser-scanning microscopy (inset) reveals thick tangle-like arrangements of non-phosphorylated neurofilaments in large pyramidal neurons. The position of the affected neuron is indicated by an arrow. The accumulation of lipofuscin is the same under control and enriched conditions. Bars 100 μm, bar (inset) 5 μm.

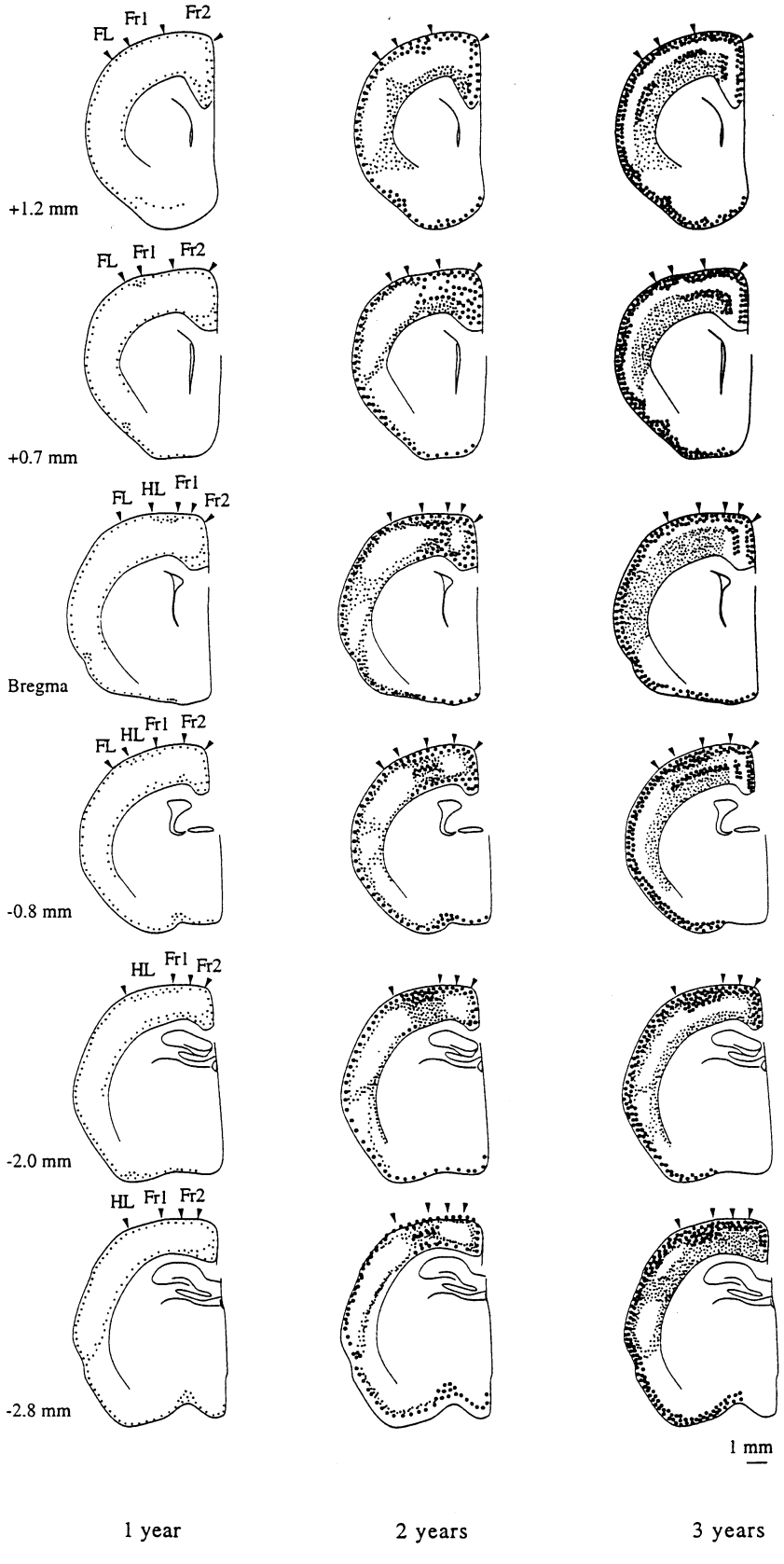

Fig. 2. Schematic drawings showing the patterns of the GFAP and lipofuscin distribution during aging. Note the prominent increase which had occurred already at the age of 2 years within cingulate, retrosplenial, fore- and hindlimb areas. Numbers give the anterior–posterior coordinates according to Zilles (1985).

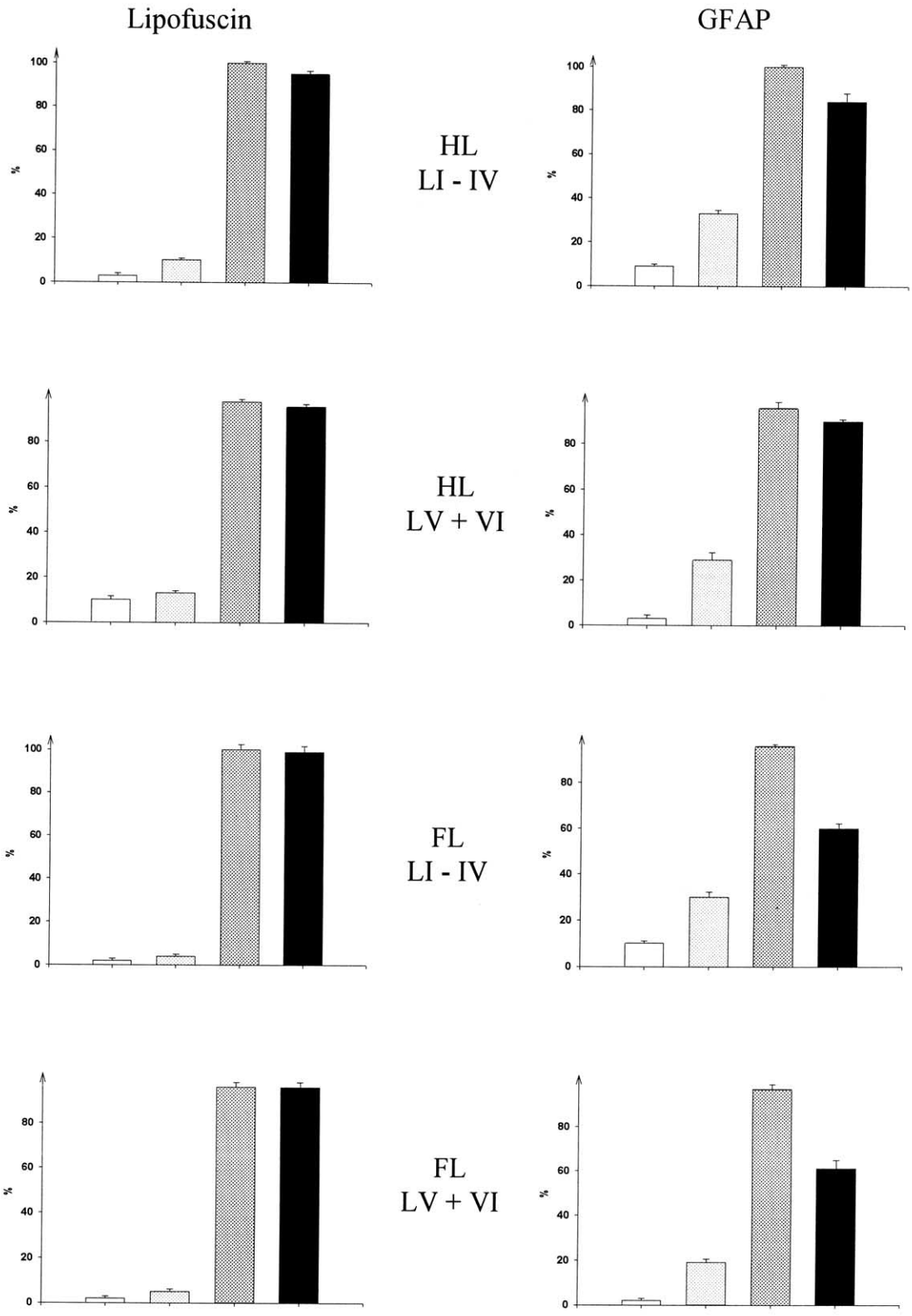

Fig. 3. Grey values of lipofuscin accumulation and GFAP expression at the age of 1 year (white column), 2 years (grey), 3 years (darkly grey) and 3 years, last 3 months in enriched environment (black), bars give the S.D.

cytes in all cortical areas (Fig. 2). At the age of 1 year, GFAP-positive astrocytes were frequently present in lamina I (L I) and associated with the pial surface (Fig. 2, left column), whereas in deeper layers positive astrocytes were mostly associated with the wall of blood vessels. Compared to younger animals (3 months of age), the number of immunoreactive astrocytes seemed to be already slightly increased at 1 year of age, since some additional weakly GFAP-immunoreactive astrocytes occurred also in cortical layers III–VI. As described above, this increase in GFAP was accompanied by a very weak, dust like autofluorescence of lipofuscin within neurons. Both neuronal lipofuscin accumulations and GFAP positive astrocytes had predominantly increased in the vicinity of cortical blood vessels. This pattern of distribution of reactive astrocytes persisted up to 3 years of age, but the number of reactive astrocytes as well as the amount of neuronal lipofuscin accumulations increased dramatically during that period (Figs. 2 and 3). Especially GFAP positive astrocytes seemed to be selectively increased in HL compared to the adjacent areas FL and Par 1. These results showed that area HL became distinctly affected by both neuronal accumulations of lipofuscin and astrogliosis (Figs. 1 and 4B and C). Despite the differences observed by visual inspection, no significant quantitative area-specific differences between HL and FL could be established for lipofuscin accumulations and GFAP expression at the age of 36 months (Fig. 3, dark grey columns). However, there were area-specific differences present within the cellular distribution pattern for lipofuscin, which was concentrated in enlarged particles within the larger pyramidal shaped neurons within layers III and V/VI of HL, whereas it was much more dispersed in the form of tiny dust like granules within the cells of FL. The most striking difference was observed when 36-month-old rats housed under standard conditions were compared with age-matched animals who had spent their last 3 months within an enriched environment. Rats housed under enriched environmental conditions showed a significant reduction of reactive GFAP positive astrocytes (HL: $84 \pm 3.8\%$, FL: $60 \pm 2.3\%$) compared to animals kept under standard conditions (100%, SD 2.5%, Fig. 3). In contrast to reactive gliosis, environmental changes had no affect on lipofuscin accumulation.

3.2. Patterns of perineuronal nets and GFAP expression

At the age of 1 year, WFA labeling indicated no perineuronal nets in cortical L I, and only a few WFA positive structures in L III of the somatosensory cortex, while marked labeling was found in L II and L IV–VI. In comparison to the somatosensory areas, the labeling of lectin-binding sites was much weaker in the MC, which represents a characteristic pattern for these cortical areas in rodents (Brückner et al., 1994). With progressing age (at 24 and 36 months) cortical L II of the HL area had lost the WFA-binding sites nearly completely. Therefore, area HL was clearly demarcated by its lack of WFA binding in L II, whereas WFA binding remained in the medially adjacent area FL (Fig. 5A and B) and the ventrolaterally neighboring area Par1. At no age WFA binding occurred in close association with GFAP positve astrocytes. WFA-binding and GFAP immunoreactivity resulted always in complementary patterns of staining. The general age-related increase in GFAP immunoreactive astrocytes resulted in a complementary reduction of WFA binding. However, a nearly complete loss of neuronal WFA binding occurred only within L II of area HL of 36 months old rats housed under standard conditions. Compared to that, in age-matched, old rats housed in the enriched environment, WFA binding within L II of area HL remained to a much greater extent (Fig. 1). Furthermore, in these animals, WFA binding sites had remained on those L II-neurons which showed the lowest amounts of tiny dust-like lipofuscin granules within area HL (Fig. 1, Lipofuscin).

3.3. Non-phosphorylated neurofilament SMI 32

Whereas, primary and secondary motor cortices (Fr2/Fr1) did not express tri-laminar patterns of SMI 32 immunoreactivity, the primary and secondary somatosensory areas showed a clear tri-laminar staining pattern including L II/III, L V and L VI in adult animals. Alterations of those patterns of SMI 32 immunoreactivities seemed to depend on the environmental conditions in which the old animals (33–36 months) were kept during the last 3 months. There was a loss of non-phosphorylated neurofilament protein related SMI 32 immunoreactivity within the hindlimb area of the cortex in rats housed under standard conditions, compared to those animals kept in an enriched environment (Fig. 1). However, some neurons of the hindlimb representation area of 36 months old rats (in enriched environment) expressed SMI 32 in unusually high amounts. At higher magnification, abnormally thick neurofilaments were detectable (Fig. 1, inset). The cortical age-related changes found for SMI 32 immunoreactive neurons remained restricted to the hind limb area when compared to the surrounding somatosensory and motor areas.

4. Discussion

Over the last two decades, the availability of rodent models has greatly aided research progress in the neu-

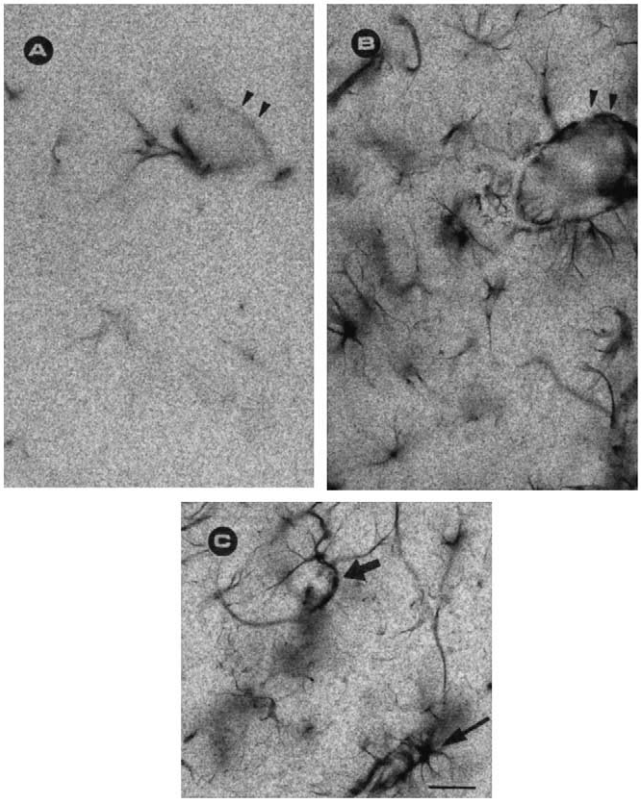

Fig. 4. Comparison of GFAP positive, blood vessel associated astrocytes (arrow heads) in FL (A) and HL (B) at the age of 3 years. The astrocytes in HL are more intensely stained and arborized (C, arrows). Bar (A–C): 10 μm.

robiology of aging (Ingram and Jucker, 1999). Rats have been the primary models of choice. In general, their behavior and their brain organization can differ decisively from that of humans. However, beyond cognitive aspects, locomotory activity in rats as a marker of vigilance and level of sensorimotor performance provides an interesting feature, that can be studied during postnatal development and aging, allowing even a comparison to age-related changes known from humans. Especially cortical lamination is basically the same in rats and humans. In the present study, we tried to correlate the appearance and severity of age-dependent cortical lipofuscin accumulation and gliosis with functional sensorimotor performance and their associated electrophysiological alterations in rats kept under different environmental conditions (Spengler et al., 1995). Furthermore, such age-dependent or lesion-induced behavioral and electrophysiological changes found in rats and humans seem to be in part reversible due to time-dependent plastic reorganization in response to electrophysiological stimulation either at the cortical level or at peripheral level (Godde et al., 1995, 1996, 2000).

The current study revealed that after 1 year clear age-dependent cellular changes occurred within the cortical area of the somatosensory hindpaw representation. At 3 years of age, we observed a strong increase of reactive gliosis. This gliosis also affected adjacent cortical areas. Since age-dependent and neuropathology associated gliosis is also commonly observed in humans (Bonte et al., 1986; Dastur, 1985; Ferszt and Cervos-Navarro, 1983; Fischer et al., 1990), the data indicate that comparable processes occur in our rat model of cerebral aging. WFA binding sites are characteristic markers for certain functional cortical and subcortical regions within the rodent and human brain (Brückner et al., 1994; Seeger et al., 1996), where they seem to be part of the extracellular matrix associated with fast spiking neuronal populations (Morris and Henderson, 2000). Furthermore, WFA binding sites develop within the cortex in relation to synaptogenesis and functional differentiation during postnatal development (Oermann et al., 1999). In contrast, WFA binding is rapidly lost by ischemic injury within cortical regions affected by synaptic stripping and injury-related astro- and microglial activation (Bidmon et al., 1997, 1998, 2001). Taken together these results indicate that WFA-binding sites are characteristic for the functional integrity of certain neuronal circuitries. Therefore, the age-dependent loss of WFA binding sites in HL may indicate a loss of functional connectivity among the affected neurons and their surrounding glial components. The forepaw area FL is expected to be a cortical area most comparable to area HL, which is clearly supported by the comparable increase in the amount of lipofuscin and reactive astrocytosis. However, FL seemed to be less affected than HL, since no loss of WFA binding was found in FL during standard housing conditions. Since WFA binding and SMI-32 immunoreactivity show almost identical cortical staining patterns in rodents (Brückner et al., 1994; Bidmon et al., 1997), it was not surprising that both WFA binding and SMI-32 immunoreactivity had decreased in HL. This may indicate that especially the loss of WFA binding and the reduction in supragranular non-phosphorylated heavy neurofilament represent a use-dependent phenomenon in HL. This difference in use-dependency between HL and FL becomes more obvious during behavioral observations from which it is well known that the forepaws are continuously used for food handling, grooming and exploration (Schallert et al., 2000). These use-dependent differences are also the basis for the interpretation of the electrophysiological changes observed for the size of receptive fields among HL versus FL, since forepaw manipulations require much more fine tuned sensory input than those of the hindlimb (Churs et al., 1996). In addition, our results point to a use-dependent reduction of reactive gliosis during enriched housing conditions for both areas FL and HL. The more pronounced reduction of gliosis in FL compared to HL probably reflects the observation that the forepaws are used more often during the exploration of new environments compared to the hindpaws. These results are in accordance with previous

findings also showing differences among the organization of the fore and hindpaw representation in the primary somatosensory cortex of adult rats induced by environmental enrichment (Coq and Xerri, 1998). The selective loss of WFA binding in HL is in correspondence with earlier electrophysiological findings showing that no representational topographic overlap occurs between areas HL and FL in our investigated rat strain (Godde et al., 1995, 1996). In addition, the data indicated that environmental use-dependent changes may be as potent as target-oriented electrophysiological stimulation (Godde et al., 1996, 2000) in reversing age- or lesion-induced degenerative processes at the cortical level since they reverse not only age-related changes characterized by electrophysiological parameters, but also reverse or stop the loss of WFA-binding and of non-phosphorylated neurofilament in combination with a reduction in reactive gliosis as revealed at the histological level.

Since age- and pathology-related lipofuscin accumulations and gliosis have been known for several decades, the observation that WFA binding sites as functional markers are also affected by these processes is new (Hilbig et al., 2000). Therefore, the future elucidation of the molecular mechanisms of this process may provide a more function related understanding of cerebral aging. Especially extracellular matrix components which are characterized by WFA-binding are known to be associated with neurons characterized by specific calcium binding proteins (Brauer et al., 1993; Härtig et al., 1992, 1999) as well as with neurons exhibiting highly specialized electrophysiological properties (Morris and Henderson, 2000). Therefore, the selective loss of WFA as well as changes in the phosphorylation of heavy neurofilaments may be useful markers for the most vulnerable neuronal circuitries within age-dependent and use-dependent neuronal degeneration. Cotran et al. (1989) have classified age-associated disorders in humans into two classes such as age-related disorders and -dependent disorders. The age-related impairments were reversible, whereas the age-dependent disorders are the direct con-

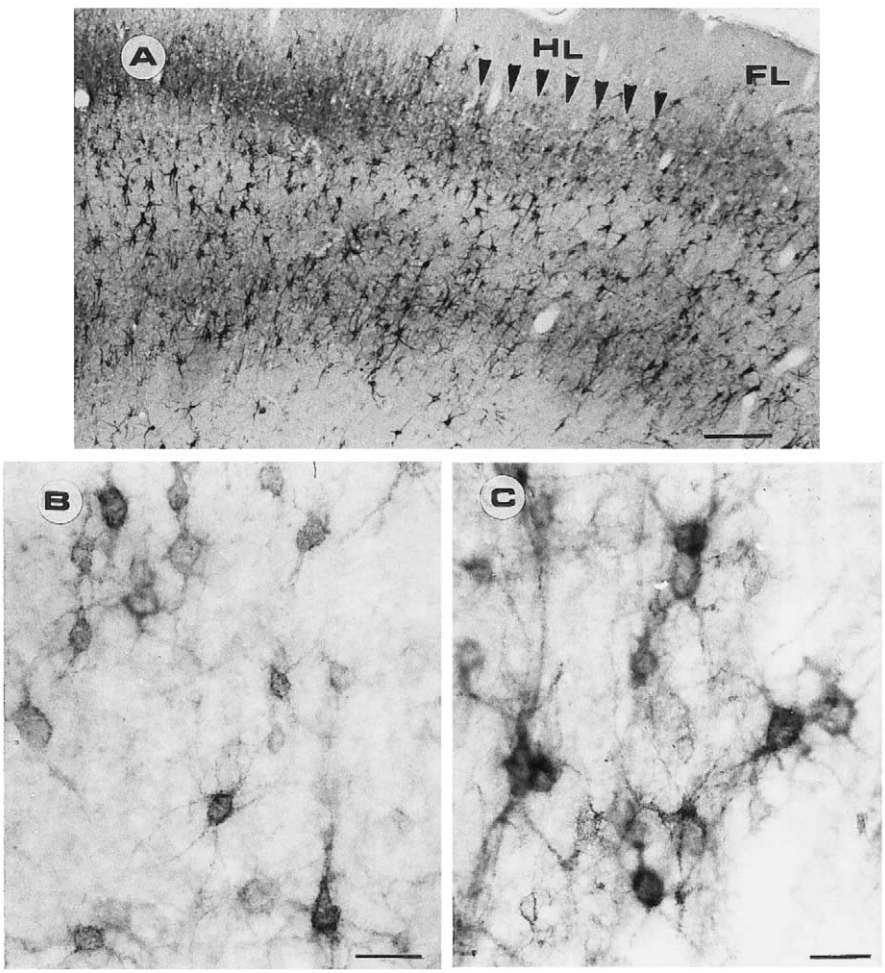

Fig. 5. Distribution of WFA-binding which labels perineuronal nets at the age of 2 years. In L II of HL (arrows), the WFA-binding is reduced and a clear border to the neighboring areas is visible (A). At the age of 3 years, only few neurons show WFA binding in HL of rats housed under standard conditions (B) whereas WFA-binding remains comparatively high in rats which had spent the last 3 months in an enriched environment (C). Bars: A, 200 μm; B,C 20 μm

sequences of physiological senescence. In the case of our FBNF1 rats, lipofuscin seems to be an age-dependent parameter, which is correlated to sensorimotor impairment but there is no causal link besides the observation that WFA-binding remained only in association with neurons showing lowest lipofuscin accumulations within HL. In contrast, age-related gliosis as well as the region-specific reduction in WFA-binding and SMI-32 immunoreactivity seem to belong to age-related, use-dependent sequelae, which were in part reversible. The current results indicate, that age-related changes occur in an area-selective manner, thereby arguing against a fairly non-specific and global nature of such changes. Specific age-related alterations in the sensory periphery have been ruled out as a simple explanation (Reinke and Dinse, 1996). The regional differences between fore- and hindpaw are especially interesting as they develop in parallel with behavioral changes. It has been shown that the described age-related changes both at a behavioral and a functional level are reversible after long-term treatment with the calcium blocker nimodipine, or that they can be substantially ameliorated by keeping the animals under enriched environmental conditions (Churs et al., 1996). The patterns of regulation of neurofilaments will reflect the functional demands on neurofilaments during the whole lifetime of neurons from differentiation in the embryo through long-term activity in the adult until aging and environmental insults lead to pathology and death. In each of these diverse neuronal states, neurofilament function will be modulated by phosphorylation–dephosphorylation reactions that define the nature of neurofilament interaction with one another and with other cytoskeletal components. A generally accepted hypothesis as to neurofilament function in axons is its role in promoting radial growth and stabilising large caliber axons, thereby increasing conduction velocity of large myelinated fibers (Pant and Veerana, 1995). That hypothesis could exclusively explain the overexpressing of non-phosphorylated neurofilaments in large projection neurons. In conclusion, the recovery of function could be caused by newly induced expression of non-phosporylated neurofilaments which resulted in an overexpression and may be in a subsequent phosphorylation of neurofilaments causing the loss of function finally. Further morphological studies are therefore under way to address the question, which cortical elements are susceptible to the beneficial effects induced by enriched environment conditions, which positively affect behavioral and electrophysiological parameters.

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