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Light and confocal laser-scanning microscopical evidences for complementary patterns of glial fibrillary acidic protein and *Wisteria floribunda* agglutinin labeled structures in human and rat brain

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Summary

We investigated the pattern of glial fibrillary acidic protein (GFAP) and *Wisteria floribunda* agglutinin (WFA) labeled structures in the superior colliculus and in the somatosensory cortex of humans and rats of different age groups using immunohistochemical methods, light and confocal laser-scanning microscopy. We never found a double labeling of WFA and GFAP positive structures neither in the superior colliculus nor in the cortex of both man and rat. The complementary pattern of WFA and GFAP labeling was present both at the macroscopic and microscopic level. We found a clear prevalence of either WFA or GFAP expression in the arborization of the astrocytes as well as in the pattern of lamination.

Introduction

Lectins such as *Wisteria floribunda* agglutinin (WFA) were known as markers of glial-neuron-interfaces and seemed to be responsible for the homeostatic acid-base regulation in the extracellular space, i.e. in the vicinity of synapses by which they could well contribute to information processing in the nervous system (CHESLER 1990; DEITMER et al. 1993; ENG 1988; KOSAKA and HEIZMAN 1989; NAEGELE and KATZ 1990). Since gliosis could interfere with the glial-neuron-interfaces in aged brains we attempt to visualize glial fibrillary acidic protein (GFAP). Expression of GFAP is an established marker for normal and reactive astrocytes (BIGNAMI and DAHL 1976; ENG and DEARMOND 1983; ENG et al. 1987; HÄRTIG et al. 1992). Reactive astrocytes express increased levels of GFAP, which allows the identification of areas which are affected by neuronal damage or degeneration and gliosis. During aging normal functions of neurons decrease and degenerative processes increase. It can be expected that changes in GFAP and WFA follow the course of gliosis. A decrease in lectin affinity for binding sites occuring during early postnatal development was reported (COOPER and STEINDLER 1986). Recently, it was reported that cortical areas abundant in extracellular matrix lectins were less affected by cytoskeletal changes in alzheimer's disease (BRÜCKNER et al. 1999). On the other hand, we found disappearing WFA clusters in the stratum griseum superficiale of the human colliculus superior when GFAP expression increased during aging (HILBIG et al. 1999). We therefore attempted to investigate the time course of lectin binding sites and of age-related gliosis in brains of old and very old man and rats.

Material and methods

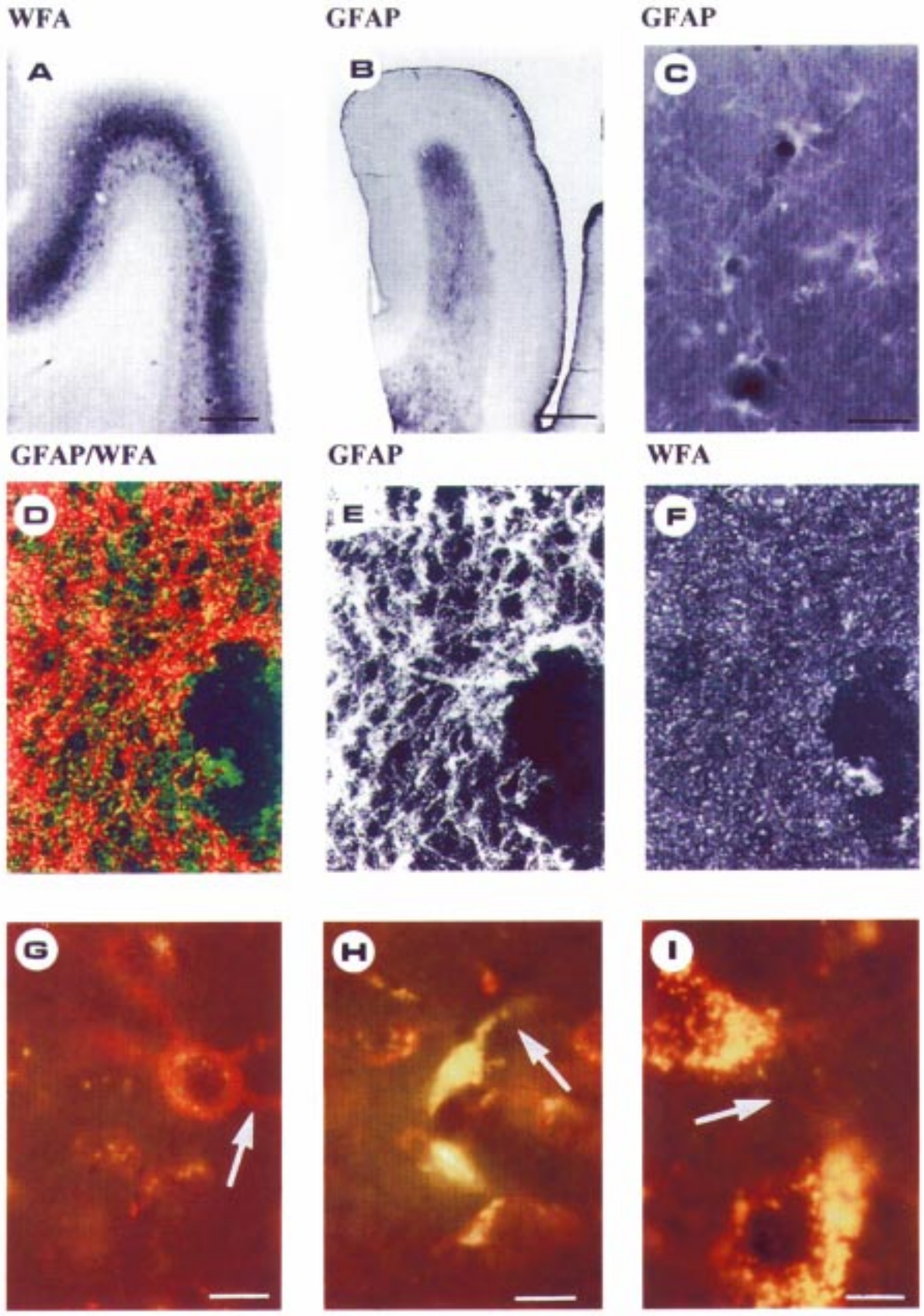
We used brain material from both humans and aged rats. All procedures were approved by the ethical committees of the University of Leipzig or Ruhr University Bochum (rats) and were within the National Institutes of Health Guide for Care and Use of Laboratory Animals (revised 1987). We used parts of five human brains of both sexes ranging in age from 56 to 81 years. After fixation in 10% formaldehyde for at least a week, the pairs of colliculi were dissected. Also the prae- and post-central gyri were identified in each hemisphere according to standard anatomical criteria (ZILLES 1990; ONO et al. 1990) and cut into 1-3 cm thick pieces in a plane orthogonal to the course of the central sulcus. Additionally, 12 FBNF1 aged rats (11 and 36 months) were anaesthetized, transcardially perfused with saline, followed by about 200 ml of paraformaldehyde in 0.1 M phosphate buffer, (PB, pH 7.4). The brains were removed and post-fixed for 2 hours in the same fixative. Subsequently the tissues of humans and rats were cryoprotected by immersion in PB containing 30% sucrose, frozen and sectioned at 30 µm. Endogenous peroxidase was blocked with 0.3% H_2O_2 for 30 min. After several rinses with PB sections were incubated overnight with biotinylated WFA (b-WFA, Sigma L-1766, Munich) at a concentration of 10 µg b-WFA/ml Tris-HCl buffer saline containing 2% bovine serum albumin (TBS-BSA). Alternate sections were incubated with the primary antibody against GFAP (monoclonal, DAKO) at a dilution 1:100, followed by biotinylated anti-mouse antibody diluted 1:50 (Vector). After several rinses both WFA and GFAP labeled sections were incubated in extravidin peroxidase (Sigma-Immunotechnicals) for one hour. Visualization of the reaction product was performed with diaminobenzidine (DAB)/H_2O_2. After rinses with PB the sections were mounted, air-dried and coverslipped with Entellan (Merck, Heidelberg).

For double labeling of WFA and GFAP we used Cy2-conjugated streptavidin (Amersham) diluted 1:50 and applied for 4 hours at room temperature. Following rinses, sections were placed in TBS-BSA and a monoclonal antibody against GFAP coupled with Cy3 (Sigma) at a dilution of 1:400 for 4°C at 12 hours. 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 of the human superior colliculus (SC) were examined in the LSM 410 confocal laser-scanning microscope (Zeiss, Germany) with excitation wavelengths at 488 and 568 nm.

Results

Under these conditions no colocalization of WFA and GFAP positive structures was found in the SC and cortices of men and rats. The complementary pattern of WFA and GFAP labeling in the human somatosensory cortex was nearly macroscopically visible (figs. 1A,B). Here, we found GFAP as a gliosis marker in the lamina I which was darkly labeled throughout all parts of the motor and sensory cortices. In contrast WFA positive cells were scattered from lamina II to V-VI. In the human SC, we found WFA labeled net-like structures reaching from the pial surface into the stratum griseum superficiale which was similar to the distribution of GFAP immunoreactivity which was not colocalized but closely associated with binding sites for WFA. Laser-scanning microscopy revealed that GFAP was present in thick astrocytic processes whereas WFA labeled the fine processes in the same or adjacent cells (figs. 1D–F). The presence of tightly packed GFAP-positive astrocytes, particularly beneath the pial surface, is suggestive of gliosis. Therefore, gliosis could mask or suppress WFA binding sites at the glial-neuron-interface. In the cerebral cortex of 36 months old rats an increase of lipofuscin in deposits (white spots in fig. 2) and GFAP occurred in similar regions whereas young rats showed clearly less lipofuscin and GFAP (figs. 1C, 2). Furthermore, in the somatosensory cortex of aged rats GFAP expression clearly differed between the cortical representational areas of the fore and hind limb since the hind limb area was demarcated by it's strong GFAP expression in cortical lamina III which was paralleled by a strong decrease in WFA binding (fig. 2). In contrast in 11 months old rats layer III of the fore and hind limb areas showed well developed perineuronal nets as detected by WFA-binding. However, even at 11 months of age small amounts of the endogenous age-dependent accumulation of lipofuscin could be found (fig. 1G, white autofluorescence of lipofuscin) which were strongly increased at 36 months of age (figs. 1H,I). It seemed that during aging the thickness of the neuronal net was negatively correlated to the amount of lipofuscin and GFAP expression in the neurons.

Fig. 1. A, B: WFA and GFAP labeling in human somatosensory cortex. Bar: 3 mm. **C:** Weak GFAP labeling in the cortex of an one year old rat, white spots represent the autofluorescence of small amounts of lipofuscin. Bar: 100 µm. **D–F:** Laser-scanning micrographs of glial structures in the human superior colliculus. Magn. 2240×, red: GFAP, green: WFA. **G, H:** WFA labeled perineuronal nets (red) in the somatosensory cortex compared to the amount of lipofuscin (white autofluorescence) in 11 (G) and 36 months (H) old rats. Arrows indicate the thickness or the complete lack of WFA nets. Bars: 10 µm. **I:** Higher magn. of a lipofuscin filled neuron with a very thin WFA labeled structure (arrow). Bar: 2.5 µm.


WFA
GFAP
GFAP
A
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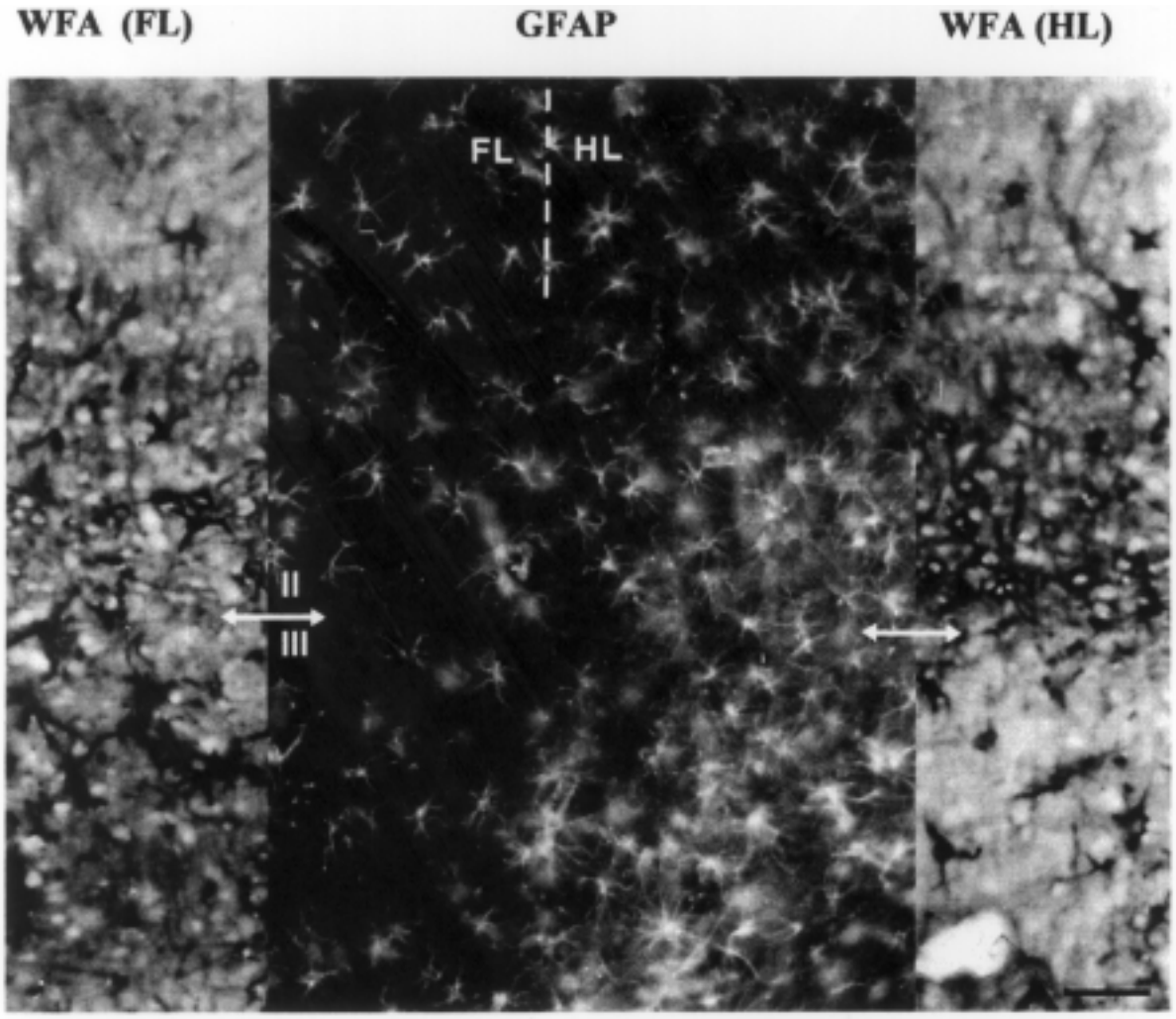

Fig. 2. Comparison of WFA and GFAP patterns of the fore limb area (FL) and the hind limb area (HL) of the somatosensory cortex in a three years old rat: GFAP expression increased in lamina III of HL whereas WFA expression decreased. Bar: 100 μm.

Discussion

In conclusion, our results support the findings of CHAO et al. (1997) that GFAP is expressed in thicker astrocytic processes whereas S-100 proteins were confined to more distal processes. Proximal and distal parts of astrocytes were involved in regeneration or reorganization of central nervous elements. In this regard WFA labels extracellularly more distal glial processes and neuronal surfaces. Additionally, WFA binding seems to depend on the function of the surrounded neuron (MULLIGAN et al. 1989; MURAKAMI 1994; MURPHY 1993; NAEGELE and KATZ 1990; SEEGER et al. 1996) as indicated by it`s loss during age-dependent neurodegeration and gliosis. Glial recognition molecules at the surface of the neuron and in the extracellular matrix form boundaries depending on the functional state and nature of the neuron (PAPPAS and RANSOM 1994; ROBELLO et al. 1994). Aging is an individual process with wide variations both in humans and rats. Especially in humans, it can depend on many factors. Recently, it was revealed in rats that decreasing levels of circulating steroid hormons may be responsible for the age-depend gliosis in various brain regions and the alterations can be partly corrected by the administration of pregnenolone (LEGRANDE and ALONSO 1998). A decrease of nerve cell profiles and simultaneous increase of GFAP immunoreactivity in the prefrontal cortex of 24-month-old rats were reported (AMENTA et al. 1994). The deteriorated cell metabolisms lead to changes of the cell membrane. This could also lead to an elimination of the lectin-binding sites on the cell surfaces of the neurons. As a result, the neuron-glia steady-state would be disturbed and gliosis is induced. Its morphological manifestation is the expression of GFAP.

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