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ORIGINAL ARTICLE

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Immunohistochemical investigations on the course of astroglial GFAP expression following human brain injury

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Abstract The course of GFAP expression by astrocytes has been immunohistochemically investigated during the first 30 weeks after human brain injury. In order to provide reliable data for a forensic wound age estimation, a quantitative morphometric analysis was performed considering the different topographic regions of the cortex as well as of the white matter. Compared to the GFAP immunoreactivity in unaltered control tissue, significantly increased numbers of GFAP positive astroglial cells could be detected adjacent to the cortical contusion from 1 day up to 4 weeks after brain injury.

Key words Brain injury · Cortical contusion · GFAP expression · Immunohistochemistry · Wound age

Introduction

Reactive gliosis, characterized by hypertrophy and proliferation of astroglial cells, is a common phenomenon in the central nervous system following tissue destruction induced by degenerative diseases or by trauma [15]. The structural changes of reactive gliosis in altered brain tissue have been considered as the major impedement to axonal regrowth after injury. On the other hand, the formation of a glial scar by activated astrocytes [13] may protect the still intact tissue from secondary lesions [19]. Since hypertrophic astrocytes are thought to play an important role in the healing phase after tissue destruction, information on the course of morphological and immunological astroglial features following brain injury might possibly contribute to a forensic wound age estimation. Numerous cellular and molecular immunohistochemical markers have been used for the morphological detection of reactive astrocytes over the last decade [19].

As the glial fibrillary acidic protein (GFAP) was found to be the major component of glial filaments [5], reactive astrocytes have been most commonly demonstrated by immunohistochemistry using specific antibodies to this protein. Increased immunostaining of GFAP has been found in several experimental models involving gliosis, such as cold lesions, stab wounds, toxic lesions and autoimmune encephalomyelitis [11].

In this study the course of astroglial GFAP expression in human brain tissue after blunt head injury has been investigated to determine whether time-related changes in GFAP immunoreactivity could provide reliable information on the age of cortical contusions.

Material and methods

Brain tissue with macroscopically visible cortical contusions was obtained at autopsy from 104 individuals, aged between 6 and 81 years (average age 44 years), who had sustained closed head injury. The survival period ranged between a few minutes and 30 weeks and the postmortem interval did not exceed 3 days. All individuals with a survival period up to 3 weeks died from cerebral dysregulation caused by neuronal damage or its secondary complications, while in the remaining cases natural causes of death (e.g. acute coronary insufficiency) were found. Secondary haemorrhages or disturbances of blood coagulation were not evident according to the clinical data. Furthermore, only those specimens which showed no signs of advanced tissue necrosis were evaluated. Cortical samples of macroscopically unaltered cerebral regions as well as brain tissue from 32 adult individuals without head injury who died of acute cardiac arrest (n = 17), traumatic asphyxia (n = 10) or carbon monoxide intoxication (n = 5) served as controls.

After fixation in 4% PBS-formaldehyde solution for a maximum of 24 h, the tissue samples were embedded in paraffin and sections (3–5 μm) were stained with hematoxylin and eosin (HE). The immunohistochemical staining was performed with a monoclonal antibody against GFAP (Dako, #M0761) according to the recommended protocols (dilution 1:100, no enzyme pretreatment, APAAP

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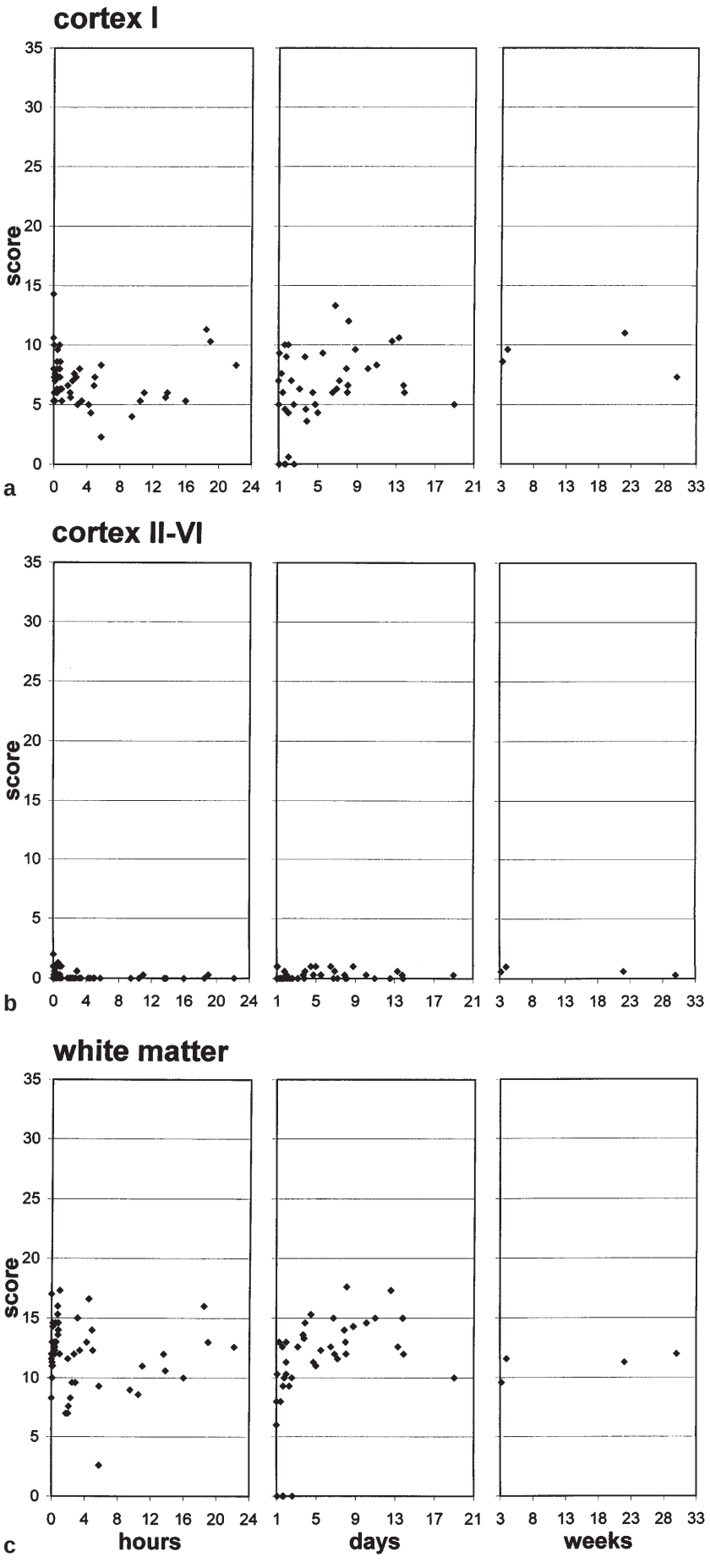

Fig. 1 a–c Average numbers (scores) of GFAP positively stained astrocytes in three microscopic fields sized 0.135 mm^2 in different topographic regions of uninjured brain tissue from individuals who had sustaind closed brain injury (n = 98). The abscissa represents the postinfliction interval in hours, days and weeks (**a**) molecular layer of the cortex (**b**) cortical layers II to VI (**c**) white matter

method). This antibody reacts with the glial fibrillary acidic protein, a 52 kDa intermediate filament protein which is present in astrocytes and some CNS ependymal cells of the human brain and spinal cord. Only those glial cells showing a distinct cellular staining reaction were regarded as positive. Specimens with signs of autolytic changes such as post-mortem cell shrinkage or diminished nuclear stainability in HE stained preparations were excluded.

Since GFAP is also physiologically expressed by astrocytes, a quantitative morphometric analysis was performed using an automatic image processing and analysis system (Leica Qwin) as described in previous studies [7, 8]. For data evaluation the total numbers of positively stained astrocytes obtained in the different cortical layers of uninjured brain regions served as intraindividual reference values and were compared to the average numbers of GFAP positive astrocytes in altered tissue. As the layers cannot generally be differentiated in damaged regions, the measuring site comprised the total area adjacent to the lesion as proposed by Takamiya et al. [20]. The following topographic regions were considered:

Area 1. Cortical area adjacent to the lesion
Area 2. Molecular layer (cortex I) of uninjured brain regions
Area 3. Cortical layers II-VI of uninjured brain regions
Area 4. White matter of uninjured brain regions

The average number of cells in three microscopic fields each 0.135 mm^2 in size (objective 16/0.40, ocular × 10) was defined as the score and the ratio (r) was calculated as the average cell number at the lesion site (area 1) divided by the average cell number in the uninjured cortex (area 2 + area 3).

Results

Uninjured brain tissue

The total number of GFAP positive astrocytes was quite different in each topographic region (areas 2-4) of unaltered brain tissue of unaltered brain tissue from individuals who had sustained brain injury. In the molecular layer of the cortex (cortex I), the average numbers of GFAP-labelled astrocytes ranged between 0.0 and 13.3, whereas in the layers II–VI of the cortex no significant GFAP expression could be detected. The maximum cell numbers were observed in the white matter with score values up to 17.6 (Fig. 1). Comparable cell numbers were found in samples from the control group. The maximum values were 12.8 (cortex I), 1.2 (cortex II–VI) and 19,8 (white matter). The

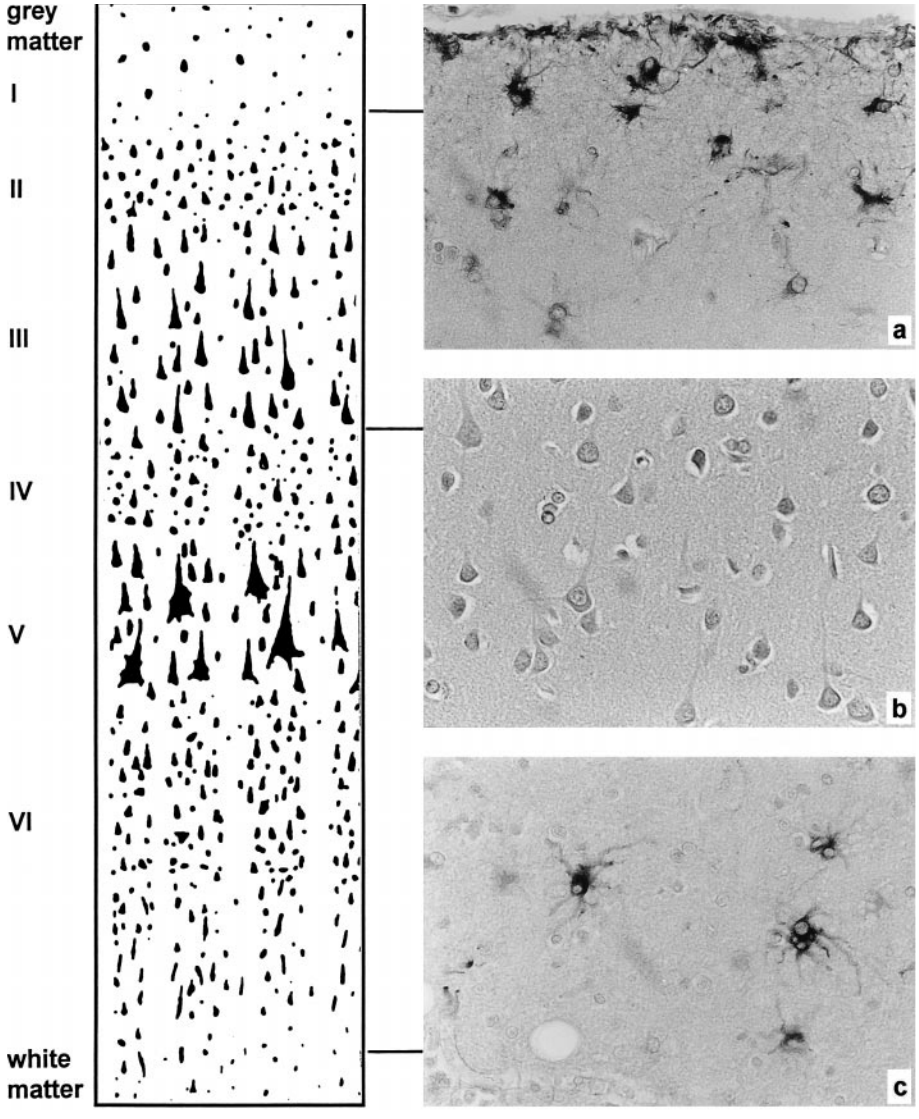

Fig. 2 a–c GFAP positive astrocytes in uninjured brain tissue (× 290) (**a**) molecular layer of the cortex (**b**) cortical layers II to VI (**c**) white matter

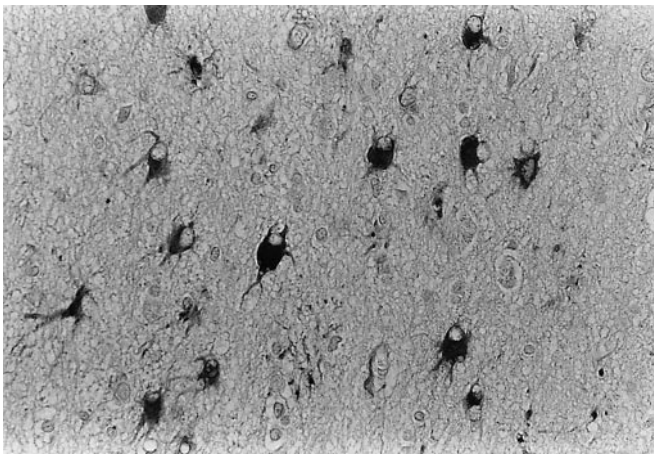

Fig. 3 GFAP labelled astroglial cells adjacent to the cortical contusion (postinfliction interval: 11 days, × 280)

astrocytes found in the white matter showed numerous fibrous processes and a distinct staining of the cell body. By contrast only a relatively small number of cell processes were visible (Fig. 2) in the majority of astroglial cells located in the grey matter (cortex I).

Cortical contusions

Out of 104 cases with cortical contusions 98 could be evaluated and the remaining 6 cases were excluded because of a significant background staining. During the first day after trauma the average numbers of astrocytes adjacent to the cortical lesion (area 1) which stained positive for GFAP did not exceed the total amount in area 2 and area 3 of the uninjured cortex. An increased GFAP expression could be found at the lesion site (area 1) 1 day after brain injury at the earliest (Figs. 3, 4). For postinfliction intervals ranging

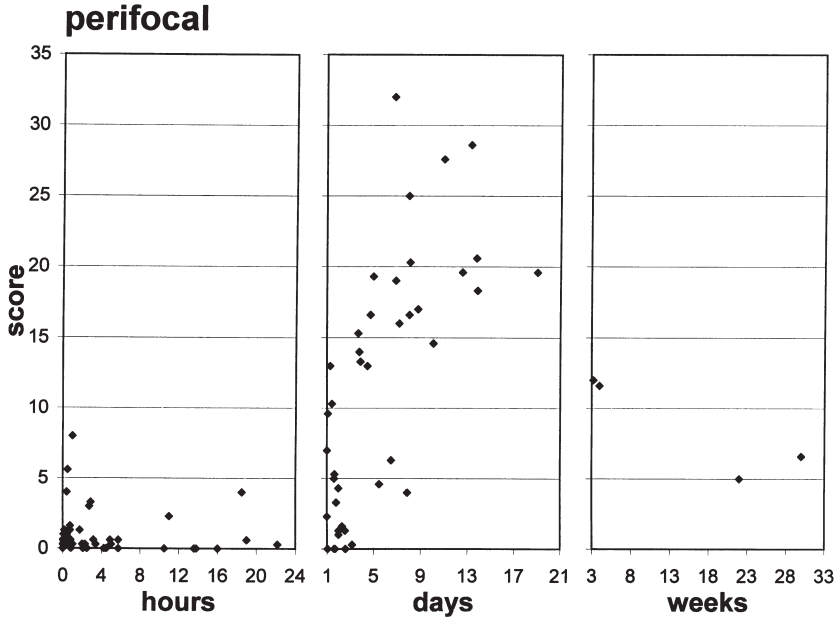

Fig. 4 Average numbers of GFAP positively stained astrocytes (scores) in three microscopic fields sized 0.135 mm^2 adjacent to the cortical contusion (n = 98) correlated with the postinfliction interval (abscissa)

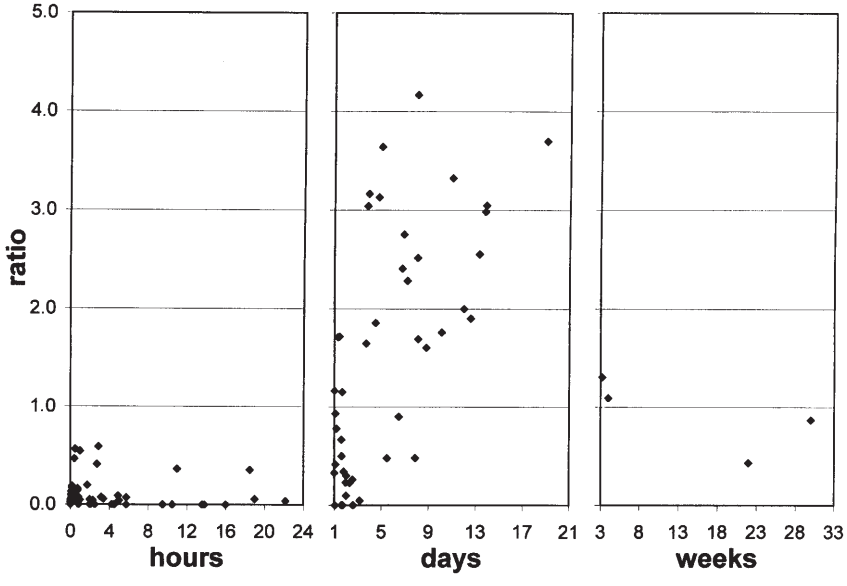

Fig. 5 Mean values of GFAP positive cells at the lesion site related to the average number of GFAP labelled astrocytes in unaltered brain regions (cortex I–VI) from the same individuals (n = 98), defined as ratio (r)

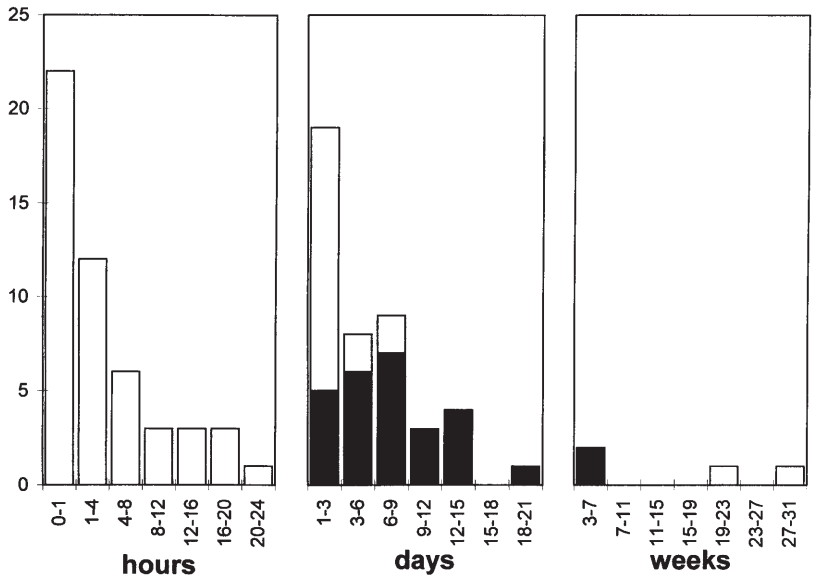

Fig. 6 Numbers of cases with positive GFAP immunostaining reaction (r > 1), related to the wound age (n = 98)

between 1 and 4 weeks, all cases showed ratios exceeding r = 1 and were therefore regarded as positive (Figs. 5, 6). In contrast to increasing numbers of cells adjacent to the cortical lesions which stained positive for GFAP, no relevant changes of GFAP expression could be observed in the tissue sections distant from the wound area, neither in the cortical layers nor in the white matter. Similarly to the astrocytes in the molecular layer of uninjured brain tissue, the majority of GFAP positive astrocytes at the lesion site were characterized by round shaped cell bodies and a relatively small number of fibrous processes (Fig. 3).

Discussion

Glial fibrillary acidic protein (GFAP) has been found to be a specific marker for differentiated astrocytes in the CNS. But in uninjured mature brain tissue, a GFAP immunoreactivity is normally observed only in fibrous astrocytes located in the white matter as well as in the molecular layer of the cortex. Protoplasmatic astrocytes, however, which are found mainly in the grey matter, have a relatively small amount of cytoplasm and a small number of glial filaments [6]. According to this unique distribution of the two types of astroglial cells in the CNS, significant differences of GFAP expression were obvious in the various topographic regions investigated in this study. In uninjured brain tissue, comparably high cell numbers were regularly found in the white matter and in the molecular layer of the cortex, whereas in the cortical layers II-VI no significant GFAP expression could be detected.

Increasing numbers of GFAP positive astroglial cells following traumatic brain injury have been described in several experimental studies in animals. Li et al. [14] found elevated numbers of GFAP positive astrocytes at the earliest 4 h after injury at the impact site in a fluid-percussion model in cats. Other authors observed an astroglial immunocytochemical response in cortical lesions after a postinfliction interval of at least 1 day [9, 11], 2 days [1, 2, 18, 20], 3 days [4, 12] or 5 days [3]. These data correlate with an elevation in GFAP gene expression (mRNA) which could be detected in response to mild cortical contusions in animals [10]. The increase in GFAP staining can result either from dissociation of glial filament bundles due to edema or due to increase in GFAP synthesis [6] by fibrous astrocytes which are thought to derive from protoplasmatic astrocytes of the gray matter (II–VI layers) without cell division [3]. On the other hand, it could be demonstrated that GFAP-containing astrocytes located in the molecular layer (I) of the cortex and the white matter have the capacity to proliferate after trauma [20]. In contrast to these experimental models, this study investigated the cell density of GFAP positive reactive astrocytes at the lesion site during the wound healing process in human brain tissue. With regard to the presence of GFAP positive astrocytes in normal brain tissue, a morphometric analysis has been performed to obtain reliable information on chronological changes in absolute cell numbers following the brain injury. During the first day posttrauma the average numbers of GFAP positive cells in the area adjacent to the damaged tissue were less than the values obtained in the cortex located far from the contusion. As the measuring fields at the lesion site comprised the total perifocal area including deeper cortical layers, which are characterized by a low GFAP content in normal brain tissue, the findings (r < 1) do not indicate reduced GFAP expression during this early period of time.

Compared to the average cell numbers in uninjured brain regions, the GFAP immunoreactivity was significantly increased 1 day after the injury at the earliest and remained elevated up to 4 weeks in all cases. The absence of immunostaining, however, must be interpreted with caution, since negative results may be caused by various external influences such as the type and duration of fixation, type of embedding medium and enzymatic pretreatment of the tissue [6]. Furthermore it has been reported that the amount

of GFAP in astrocytes of adult mice, rats and humans increases gradually with age [16, 17]. With regard to this physiological increase the GFAP expression detected at the lesion site must be compared with the level of GFAP immunoreactivity in unaltered brain regions from the same individual. Taking these aspects into consideration it can be concluded that significantly increased numbers of GFAP positive astrocytes adjacent to the cortical contusion indicate a wound age of at least 1 day.

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