ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Review Acta Neurobiol Exp 2008, 68: 155–168

Spatiotemporal dynamics of astroglial and microglial responses after photothrombotic stroke in the rat brain

Dorota Nowicka[1]*, Karolina Rogozinska[1], Monika Aleksy[1], Otto W. Witte[2], and Jolanta Skangiel-Kramska[1]

[1]Laboratory of the Molecular Basis of Brain Plasticity, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland, *Email: d.nowicka@nencki.gov.pl; [2]Klinik fur Neurologie, Friedrich Schiller University Jena, Jena, Germany

The effects of photothrombotic stroke in primary somatosensory cortex on astroglial and microglial activation in various regions of lesioned brain were examined at different time points, using immunohistochemistry and lectin binding. The increase in GFAP expression was observed exclusively in the ipsilateral hemisphere, both in the perilesional area and cortical regions distant from the infarct. This remote increase was detectable up to sixty days after the infarct. Transient GFAP elevation was also found in the hippocampus one day after photothrombosis, whereas it was more prolonged in amygdala, as demonstrated at four days after lesion. In contrast to a widespread astrocytic activation, the microglial response was short-lasting and local, confined to lesion and perilesional area. Widespread and prolonged activation of astrocytes after stroke may provide factors promoting slowly developing recovery processes in the whole brain, while microglial response seems to be involved in local repair and removal of cellular debris.

Key words: GFAP, IB4, photothrombosis, astrocytes, microglia

INTRODUCTION

Cerebral ischemia produces complex molecular and functional changes in the brain. Among them are strong astroglial and microglial activations. Astrocytes are crucial to the normal function of the central nervous system. They provide neurons with energy substrates and produce precursors of neurotransmitters (Forsyth 1996, Kirchhoff et al. 2001). They are also important in neuronal antioxidant defense, secrete neuroprotective and neurogenic factors and are implicated in maintaining the homeostasis of the extracellular milieu following ischemia (Nakata et al. 1993, Kirchhoff et al. 2001, Song et al. 2002, Haupt et al. 2007). On the other hand, astrocytes can contribute to the progression of damage of neurons by releasing glutamate in glutamate- and calcium-dependent manner and thus, to progression of ischemic lesion (Anderson et al. 2003). Moreover, the inevitable consequence of ischemic infarct is glial scar formation, which, in the long term, prevents the axonal growth and impedes recovery (Fawcett and Asher 1999, McGraw et al. 2001).

Microglia are brain immune cells. In non-pathological environment microglia are ramified cells with small body, distributed evenly throughout the brain. Upon activation the cells shorten their processes and develop first stellate and then ameboid forms, which are morphologically and immunohistochemically indistinguishable from blood macrophages. Upon pathological conditions, they can exert many functions by releasing cytokines, chemokines and cytotoxins or by transforming into ameboid forms that can phagocyte cells or cellular debris (Giulian et al. 1993a, Raivich et al. 1999, Gregersen et al. 2000). The role of microglial cells in the development of ischemic insult is not yet clarified. There is a considerable body of evidence suggesting that, apart from beneficial effects, the pro-inflammatory action of microglia can be detrimental for the injured brain and contribute to infarct

Correspondence should be addressed to D. Nowicka, Email: d.nowicka@nencki.gov.pl

Received 20 June 2007, accepted 15 March 2008

evolution (Giulian et al. 1993ab, reviewed in Stoll et al. 1998).

The glial response to stroke has been widely studied in a plethora of animal models including variations of focal permanent or transient middle cerebral artery occlusion (MCAO) and photothrombosis (Morioka et al. 1993, Schroeter et al. 1999, 2002, Schilling et al. 2003, Wang and Waltz 2003, Durukan and Tatlisumak 2007). Most studies, however, concentrated on the areas localized close to the injury. Less effort has been done to evaluate glial reaction in areas remote to the infarct. Schroeter and colleagues (1995) showed increased glial fibrillary acidic protein (GFAP) expression in cortical areas remote to the lesion site, located laterally to the lesion. Similarly, Yamashita and others (1996) revealed increased GFAP mRNA levels in some non-ischemic areas after permanent MCAO. Schroeter and coauthors (1999) revealed the activation of microglial cells in the cortical areas ipsilateral to photochemically induced stroke. In all cases, however, the analyzed area was within few millimeter zone around the infarct core. Moreover, only few studies addressed glial reaction at time points later than 7 days after the infarct. Taking into account the possible role of glia in ischemic pathology, it seems of a great importance to investigate the range and severity of gliosis in the brain. In this study we attempt to determine the spatiotemporal course of astrocytic and microglial reaction after focal cortical stroke in rats. For this purpose the photothrombotic stroke model was used as a method to induce a reproducible focal injury in the cerebral cortex (Watson et al. 1985). To reveal astrocytes and microglia in various cortical and subcortical brain regions at different time points (from 4 h up to 60 days) after induction of ischemia we used immunohistochemistry and lectin binding, respectively. GFAP staining was used to trace the astrogliotic response since in the adult normal (non-pathological conditions) cerebral cortex only about 5% of normal astrocytes have detectable levels of GFAP (Ludwin et al. 1976, Bignami and Dahl 1977, Savchenko et al. 2000). In pathological conditions in the brain, some astrocytes proliferate and change their characteristics by intensified GFAP synthesis and positive immunostaining (Davies et al. 1998, Monzon-Mayor et al. 2000). Thus, increase of expression of GFAP is a sensitive indicator for the degree of reactive transformation in the brain. To label microglia we used isolectin B_4 (IB_4) from *Griffonia simplicifolia*, binding to the molecule of α-D galactose of microglial glycocalyx (Streit and Kreutzberg 1987). The lectin recognizes both resting and activated microglial cells, as well as ameboid forms.

METHODS

The experiments were performed on 54 male Wistar rats (weighing 250–280 g); all procedures were performed in accordance with the European Committee Council Directive of 24 November 1986 (86/609/EEC) and the experimental protocol was approved by the Local Ethics Committee.

Photothrombosis

For inducing the infarct, we used the photochemical procedure described by Watson and others (1985). Rats were anaesthetized with isoflurane via Stoelting's gas anesthesia face-mask attached to stereotactic frame. The animals were breathing spontaneously and during the operation the temperature of the body was kept at 37°C using a self regulating heating pad. A catheter was inserted into the left femoral vein, and the scalp was incised for exposure of the skull surface. A fiber optic bundle (aperture 1.5 mm) of a cold light source (KL 1500LCD, Germany), was placed straight on the skull surface on the right hemisphere 4.5 mm posterior to bregma and 4 mm lateral to the midline position (i.e. aiming the barrel cortex). Following the onset of illumination, 0.4 ml of the photosensitive dye Rose Bengal (10 mg/ml, Aldrich Chemical Company, Milwaukee, WI, USA) was infused through a catheter into the femoral vein. The brain was then illuminated through the intact skull for 20 min. Afterwards, the catheter was removed, the wounds were sutured and rats were allowed to awaken and returned to their cages.

Tissue processing for single GFAP immunofluorescence and Nissl staining

After 4 h (n=5), 1 (n=5), 4 (n=5), 7 (n=5), 28 (n=5) and 60 days (n=4), rats were decapitated in deep Nembutal (100 mg/kg i.p.) anesthesia. As controls we used sham-operated (that received saline and light), weight matched rats (n=7). These rats were decapitated 7 days after sham operation. The brains were

removed and immediately frozen on dry ice. Serial coronal sections were cut at 20 μm in a cryostat and mounted onto poly-L-lysine-coated slides). Every third section was chosen for Nissl staining to reveal histology of the infarct and other areas. Brain areas were identified according to Paxinos and Watson (2005). For GFAP immunofluorescence, the sections were fixed in ice cold 4% paraformaldehyde in PBS, rinsed 3 times in PBS containing 0.4% Triton X-100 (PBS-T). The following steps were performed according strictly to manufacturer's protocol. Nonspecific binding was blocked by incubating sections for 1 hour with M.O.M. Blocking Reagent from Vector M.O.M. Kit for monoclonal antibodies (Vector Laboratories, Burlingame, CA, USA). The sections were then washed shortly 3 times in PBS-T. Then the sections were pre-incubated with M.O.M. Diluent for 5 min at RT. The excess of the diluent was tapped off and mouse monoclonal anti-GFAP (Sigma, Poznan, Poland) was applied on sections diluted 1:1 000 in M.O.M. Diluent. After half hour incubation at room temperature and three 2 min rinses in PBS, sections were incubated with M.O.M. biotinylated antibody for 10 min. After further rinses, avidin-fluorescein conjugate (Vector Laboratories, Burlingame, CA USA, 1:100) was applied onto the sections for 20 min. Finally, the sections were washed again 3 times 5 min in PBS, dried and mounted in Vectashield Mounting Medium for fluorescence (Vector Laboratories, Burlingame, CA USA).

Tissue processing for double staining for GFAP and microglia

As IB_4 did not work on fresh frozen sections, separate group of animals was prepared for double staining. Animals were sacrificed by transcardial perfusion with 4% paraformaldehyde in PBS after 1 (n=3), 4 (n=3), 7 (n=3), 28 (n=3), 60 (n=3) days post lesion. Three sham operated animals perfused 7 days post operation served as control. The brains were removed and immersed in the same fixative for 24 hours. Serial coronal sections of 20 μm were cut in a cryostat and mounted onto poly-L-lysine-coated slides. The sections were dried, additionally fixed in 4% paraformaldehyde for 5 min, washed and incubated with biotinylated *Griffonia simplicifolia* isolectin B_4 (Sigma, Poznan, Poland, 1:1 000) overnight at 4°C. Next day, the sections were washed 3 times 5 min in PBS and incubated with streptavidin-fluorescein conjugate (Vector, 1:100) for 20 min, washed and subjected to the same protocol of GFAP immunofluorescent staining as described above, except for secondary antibody, which this time was directly conjugated with Texas Red (Vector Laboratories, Burlingame, CA, USA, 1:200). The sections were observed in Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). Images were obtained using Image Pro Plus software (Media Cybernetics, Silver Springs, MD, USA).

Control of immunolabeling specificity

In fluorescent labeling assays the specificity of staining was verified in two ways, first by omission of the primary antibodies, second, by omission of secondary antibodies. Both tests resulted in disappearance of fluorescent staining.

Image capturing and figure preparing

The images were captured under 20× objective using Nikon Eclipse 80i fluorescent microscope equipped with a CCD camera and analyzed in Image Pro Plus software. Sections from the level of striatum (areas rostral to infarct, 0.2 mm from Bregma), dorsal hippocampus (infarct level) and visual cortex (areas caudal to infarct, −5.8 from Bregma) were chosen for analysis. Eight sections from each animal, representing each level studied were visually inspected. Figures were prepared using Adobe Photoshop 7.0. Images were compiled in panels and elaborated simultaneously to improve brightness and contrast.

RESULTS

General pathological changes and astrocyte activation in the infarct core and boundary

The animals recovered well from surgery and anesthesia. In sham operated animals numerous GFAP-positive astrocytes were present in all layers, although with various densities. In layer IV the GFAP-positive cells were sparser when compared to supragranular and infragranular layers. Some individual variability between animals in numbers of GFAP-positive cells was observed (data not shown). As soon as four hours after photothrombosis a large

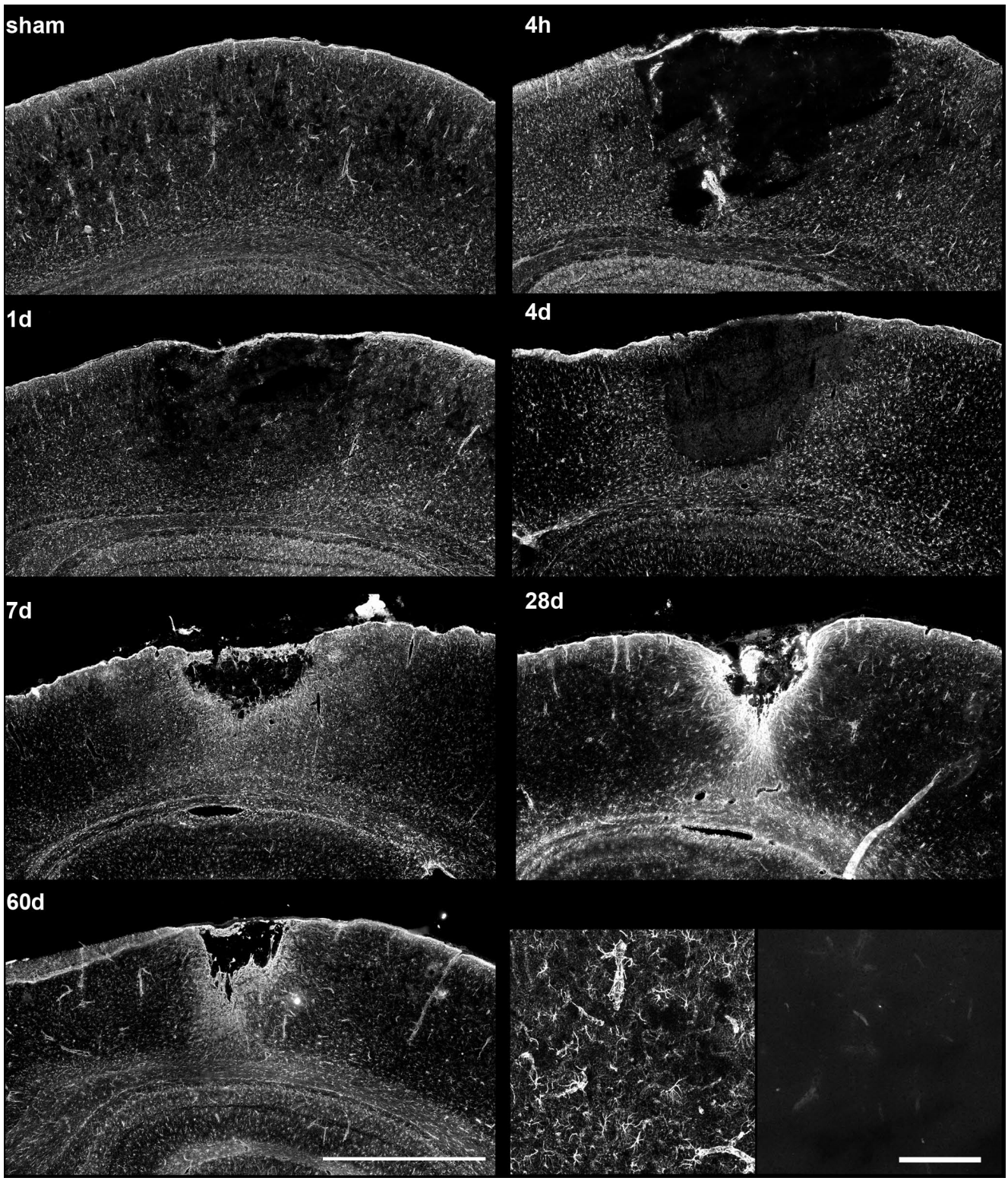

Fig. 1. Astrogliotic response at the level of the infarct. Note a dramatic decrease of immunostaining in the lesion core 4 hours after the infarct. A wide zone of reactive astrocytes starts to form around the lesion as soon as 1–4 days after the lesion and results in glial scar observed 28 and 60 days post stroke. In the lower right corner there are two pictures showing details of GFAP immunofluorescence from contralateral cortex (left) and the core of the lesion (right) 4 hours after photothrombosis. Large scale bar is 2 mm, small scale bar is 250 μm.

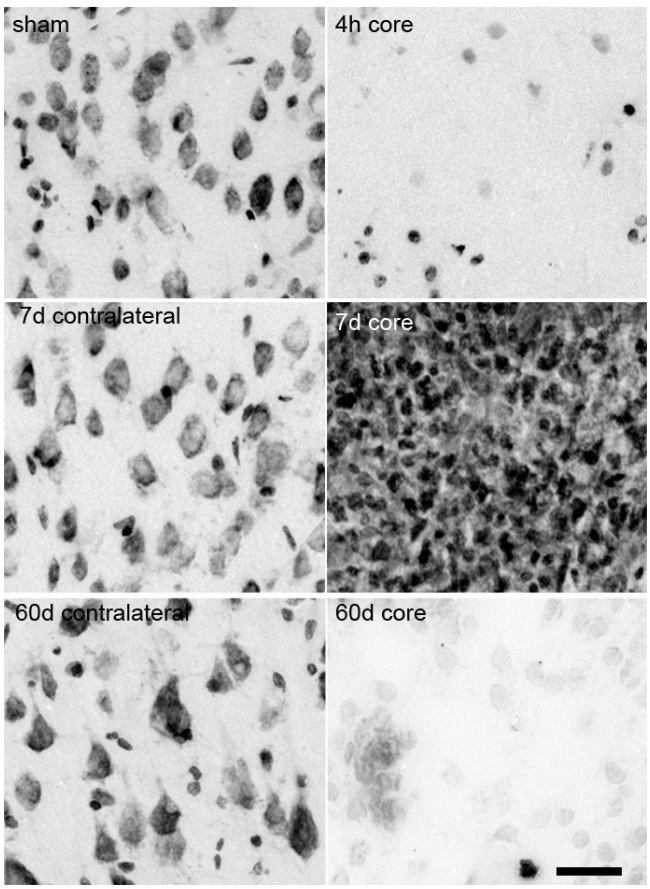

Fig. 2. Representative photomicrographs of cortical layer IV of cresyl violet stained sections from sham – operated and photothrombosis – subjected rat brains (4 h, 7 and 60 days post lesion). As soon as 4 hours post lesion a prominent loss of cells can be observed. At day 7 the lesion is filled with strongly stained cells, presumably microglia, whereas at day 60 only few weakly stained cells are visible. Note that 7 and 60 days after the lesion the staining appears normal on the contralateral side. Scale bar is 60 μm.

conical lesion spanning through all cortical layers, but not reaching corpus callosum, was observed (Fig. 1). Nissl staining revealed a massive loss of cells within the lesion. Only few cells, most of them with small dense nuclei, were still detectable by Nissl stain (Fig. 2). At this time point the core of the lesion was already almost entirely devoid of GFAP immunofluorescence (Fig. 1). No increase in GFAP expression was observed in the perilesional tissue compared both to contralateral cortex of the lesioned brain and to ipsilateral cortex of sham operated rat. One day post lesion a marked increase of GFAP immunofluorescence could be visible close to the infarct (Fig. 1) GFAP-positive astrocytes were forming a wide rim (1–1.2 mm) around the lesion core. In most animals a local edema was seen at that time in the area of infarct (Fig. 3). In other the swelling was definitely less distinct (Fig. 1). At day 4 the rim became more pronounced (Fig. 1). Seven days post stroke Nissl stain revealed the core of the lesion densely packed with darkly stained cells. The GFAP immunofluorescence next to the infarct increased and further reduced in width starting to form a glial scar. At later stages (28 and 60 days post lesion) the enhanced GFAP expression confined to a thin layer of morphologically polarized astrocytes located close to the boundary of the injury, corresponding to glial scar. The branches of the cells were arranged parallel to each other and perpendicularly to the infarct boundary. Together with glial scar formation, a considerable shrinkage of lesion core was observed (Fig. 1), confirming the results of Shanina and coauthors (2005).

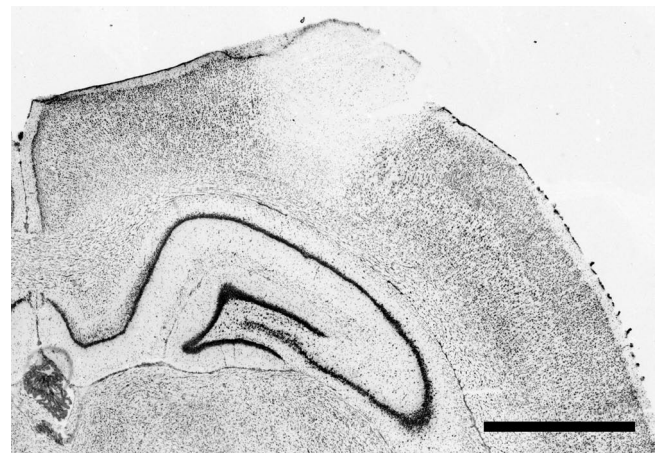

Fig. 3. A representative example of Nissl-stained section from rat sacrificed 24 h after photothrombotic infarct. Notice lack of staining in the infarct core and substantial but local edema in the ipsilateral hemisphere. Scale bar is 2 mm.

Remote areas

Elevation of GFAP expression was observed in almost entire ipsilateral hemisphere. A considerable increase of GFAP immunofluorescence in remote cortical areas was first observed as soon as 4 day post lesion (Fig. 4). In motor and somatosensory areas of frontoparietal cortex (FrPaM and FrPaSS) 0.2 mm from Bregma the increase could be observed throughout all thickness of the cortex, although the most pronounced effect was found in layers IV-VI (Fig. 4). Four and 7 days after the lesion the GFAP expression in the

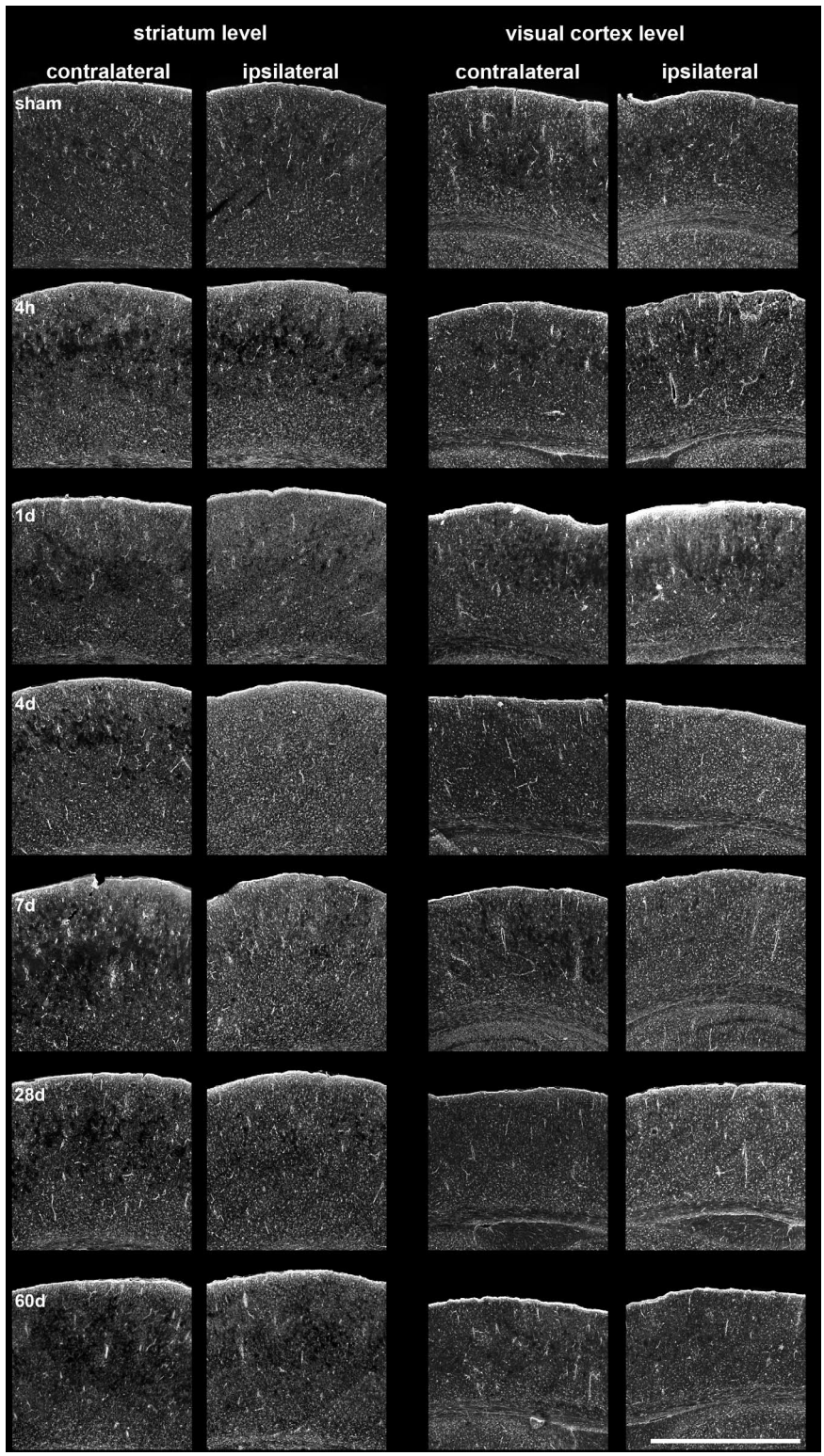

Fig. 4. GFAP immunoreactivity in coronal sections of rat brains subjected to photothrombotic stroke 4 h, 1, 4, 7, 28 and 60 days after the lesion (representative examples). Left two columns illustrate sections from the level of striatum and frontal cortex. Note the increase of GFAP immunostaining starting 1 day post-stroke and still persisting after 60 days. Right two columns illustrate GFAP immunoreactivity at the level of visual cortex. The most pronounced effect is observed 4 and 7 days post lesion. Scale bar is 2 mm.

ipsilateral FrPaM and FrPaSS cortices reached maximum (Fig. 4). Twenty eight days after photothrombosis differences between ipsilateral and contralateral cortices were still visible, particularly at the level of striatum (Fig. 4). The influence of photothrombotic infarct was also observed in cortical areas posterior to stroke (Bregma −5.8). These areas corresponded to primary and secondary visual cortices. The most profound effect was observed 4 and 7 days post lesion (Fig. 4). No changes in GFAP level in homotopic areas of the contralateral, unlesioned cortex were observed (Fig. 4) as well as no apparent histological aberrations were found in these areas (Fig. 2).

Astrocytic GFAP response was observed also in other brain structures, especially in limbic areas. In amygdala a prominent increase was observed. The increase could be clearly distinguished in lateral and basolateral nuclei. This effect was transient and the maximum expression was observed 4 days post lesion (Fig. 5). Less distinct, but still clearly visible increase of GFAP expression was observed in ipsilateral hippocampus, 1 day after stroke (Fig. 6). The astrocytosis was observed in all layers of the hippocampus; however the strongest GFAP immunofluorescence was present directly beneath the infarct core (Fig. 6). Up to day 4 the activation of GFAP expression declined and was observed only in upper layers of CA1. Later, the pattern and intensity of immunofluorescence returned to control levels. We did not notice any astrocytic activation in the contralateral hippocampus at any of time points examined (not shown).

Microglia activation

In contrast to astrocytic activation, microglial response was local, confined to the core and neighboring perilesional cortex. First activated IB_4-positive cells were observed in the infarct core within 24 hours after photothrombosis (Figs 7, 8). They were oval in shape and localized within the infarct core, but close to its inner boundaries. These cells probably infiltrated the core from peri-infarct cortex as few microglial cells were observed near the lesion compared to remote cortical areas (Fig. 8). On day 4 the microglial reaction had intensified. Isolectin IB_4 binding delineated numerous ameboid and round cells accumulating in the infarct core. At this time point no spatial overlapping between activated astrocytes and microglia could be observed as no IB_4-positive were visible in areas adjacent to the boundary, occupied by reactive astrocytes (Figs 7, 8). From day 7 ameboid cells were present throughout the entire lesion in the infarct zone

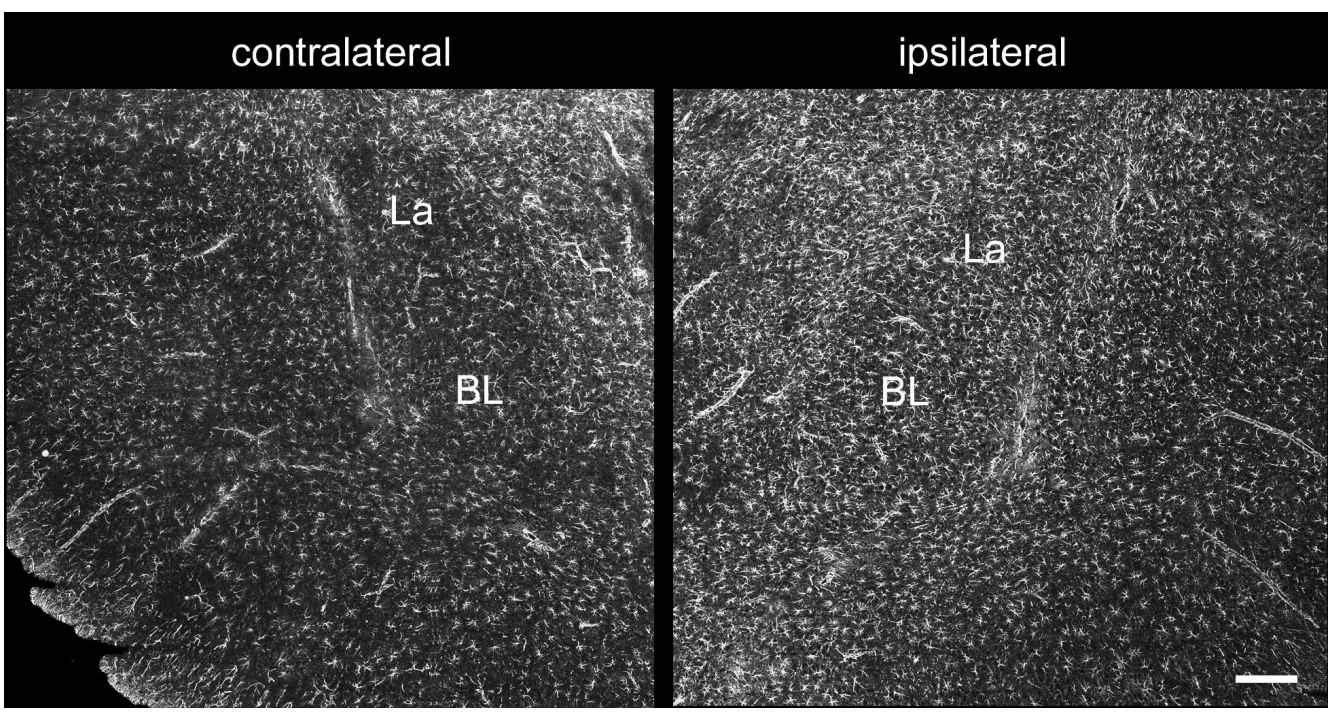

Fig. 5. Immunoreactivity for GFAP in amygdalar nuclei 4 days post lesion. GFAP was elevated in ipsilateral amygdala. (La) lateral nucleus; (BL) basolateral nucleus. Scale bar is 200 μm.

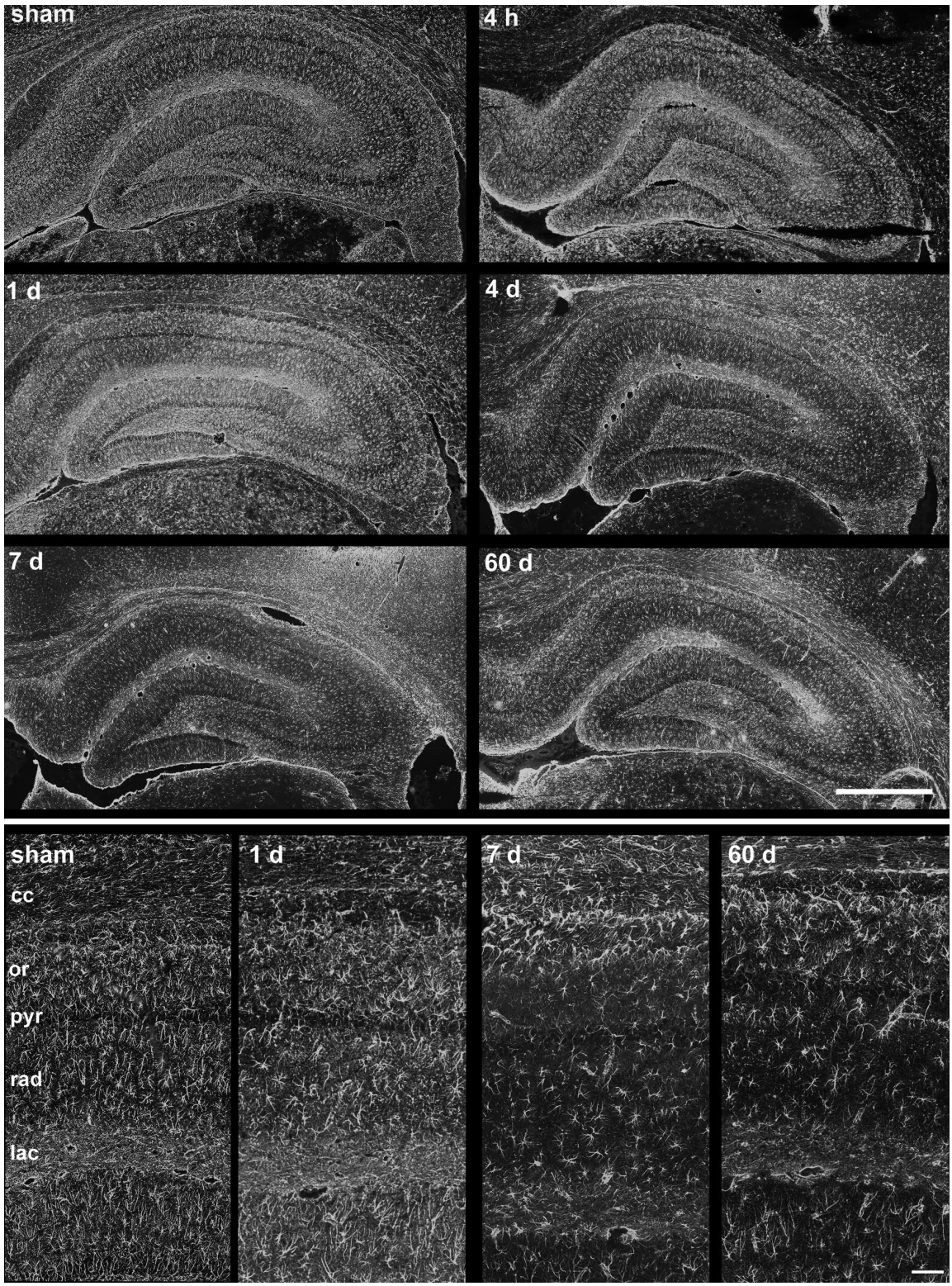

Fig. 6. Changes in GFAP immunorectivity in the ipsilateral hippocampus after different survival times after stroke. (Upper panel) after 4 hours no obvious alterations in GFAP immunoreactivity was observed. Note strong increase in immunostaining 1 day post stroke. The effect was transient and disappeared 4 days later. (Lower panel) high power microphotographs of CA3 field show details of GFAP increase. (cc) corpus callosum; (or) stratum oriens; (pyr) stratum pyramidale; (rad) stratum radiatum; (lac) stratum lacunosum moleculare. Scale bars are 500 μm (upper panel) and 100 μm (lower panel).

(Figs 2, 7, 8). Around the lesion highly activated stellate microglial cells, mingled with glial scar-forming astrocytes were observed (Fig. 8). At later stages (28 and 60 days post lesion) the microglial reaction declined. Only few, single ameboid cells could be discerned within the mass of entangled blood vessels. Microglial reaction was also present along callosal fibers (Fig. 7) reflecting secondarily degenerating fibers tracts.

DISCUSSION

In this study we further characterized a photothrombotic model of ischemic stroke in rats. We found that massive astrocytic response developed not only in vicinity of the lesion but also in distant areas from the infarct. The reactive astrocytes were observed in remote frontal cortical regions (at the level of striatum) and in caudal region (at the level of striate cortex). Beside neocortical areas other structures such as hippocampi and amygdalae showed a transient increase in GFAP immunoreactivity. The spatiotemporal pattern of reactive astrocytes after the pial vessel disruption model of irreversible focal ischemic lesion in rat was investigated by Wang and Waltz (2003) up to day 6 after injury. This study focused on area close to the lesion and areas more distal to the lesion that represented so called remote gliotic response. In fact, these distal areas were only examined 1mm lateral to the boundary of the lesion. Schroeter and others (1995) showed the increased expression of GFAP beginning 3 days after photothrombosis, persisting after 6 days and disappearing 14 days post infarct. Again, in this study, the analyzed areas were localized lateral to the lesion core, at the level of infarct only.

There are some studies showing contralateral effect of photothrombotic stroke in the cortex. For example, Jablonka and Kossut (2006) indicated that after the stroke the intact hemisphere shows higher metabolic activation in several cortical regions. Interestingly, we did not find any changes in GFAP level of the contralateral, unlesioned side, in contrast to studies of Bidmon and colleagues (1998), who observed some increase in GFAP staining in homotopic areas of the contralateral cortex. The authors state that the increase they observed was merely "slight", despite substantial injury. One possible explanation of these discrepancies is that, in our conditions, the infarct was substantially smaller, so corpus callosum was spared and commissural fibers were not affected by photothrombosis. It is plausible that the contralateral effect of photothrombotic stroke is highly dependent on the infarct size and depth.

Besides, the contralateral effect may be due to edema and changes in intracranial pressure rather, than to the infarct in the ipsilateral cortex (Witte et al. 2000). In our experiments the edema was very local. We suppose that the impact of the edema on the contralateral cortex was not strong enough to elicit GFAP increase. Moreover, we showed that photothrombotic stroke did not change the level of D1 dopamine receptor binding sites in the homotopic contralateral cortex (Rogozinska and Skangiel-Kramska 2005). In other studies the contralateral cortex served directly as reference area in brains subjected to focal stroke (Schroeter et al. 2001, Yao et al. 2005, Rojas et al. 2006).

Little attention had been given to the ischemic insult-induced astrocytic reaction in other brain areas. Olson and McKeon (2004) described elevation in GFAP levels in the contralateral hippocampus 3 days after unilateral hypoxia/ischemia. We showed transient but substantial increase in GFAP expression in the ipsilateral hippocampus one day after the stroke. This discrepancy results probably from differences in experimental model employed. In our experiments, astrogliotic reaction in the hippocampus was rather the result of an edema observed within lesioned hemisphere, than direct ischemic insult of hippocampal area. It was short-lasting and not accompanied by microglial activation. In the case of hypoxia/ischemia model, the contralateral effect was secondary, probably triggered by loss of axonal projections to the contralateral hippocampus.

To our knowledge, no studies had been done on the effects of cortical photothrombotic stroke on amygdalar expression of GFAP. We showed a marked increase in GFAP level in lateral and basolateral nuclei 4 days post lesion. There is evidence, however, that, in MCAO model, ischemia activates gene expression in amygdala. Allen and coauthors (1995) and Cheung and others (1995) showed that neuropeptide Y staining was significantly increased in the basolateral nucleus of the amygdala, ipsilateral to the middle cerebral artery occlusion. Moreover, corticotropin-releasing hormone mRNA was also up-regulated in the amygdala, 60 min following MCAO (Wong at al. 1995). We presume that the observed increase in GFAP expression is due to activation of amygdalar nuclei by massive inputs from insular and parietal cortices (McDonald 1998), as these

cortical areas are strongly activated by spreading depression. Interestingly, we did not observe any increase in GFAP immunofluorescence in thalamic nuclei although various effects, such as neurodegeneration or activation of gene expression were observed by other groups (Herrera and Cuello 1992, Figueiredo at al. 1995, Sulejczak et al. 2004). The lack of thalamic reaction is probably attributable to small lesion size, sparing callosal fibers and not resulting in retrograde degeneration of thalamocortical axons.

The mechanism of astroglial activation in remote brain areas is not quite elucidated. Schroeter and col-

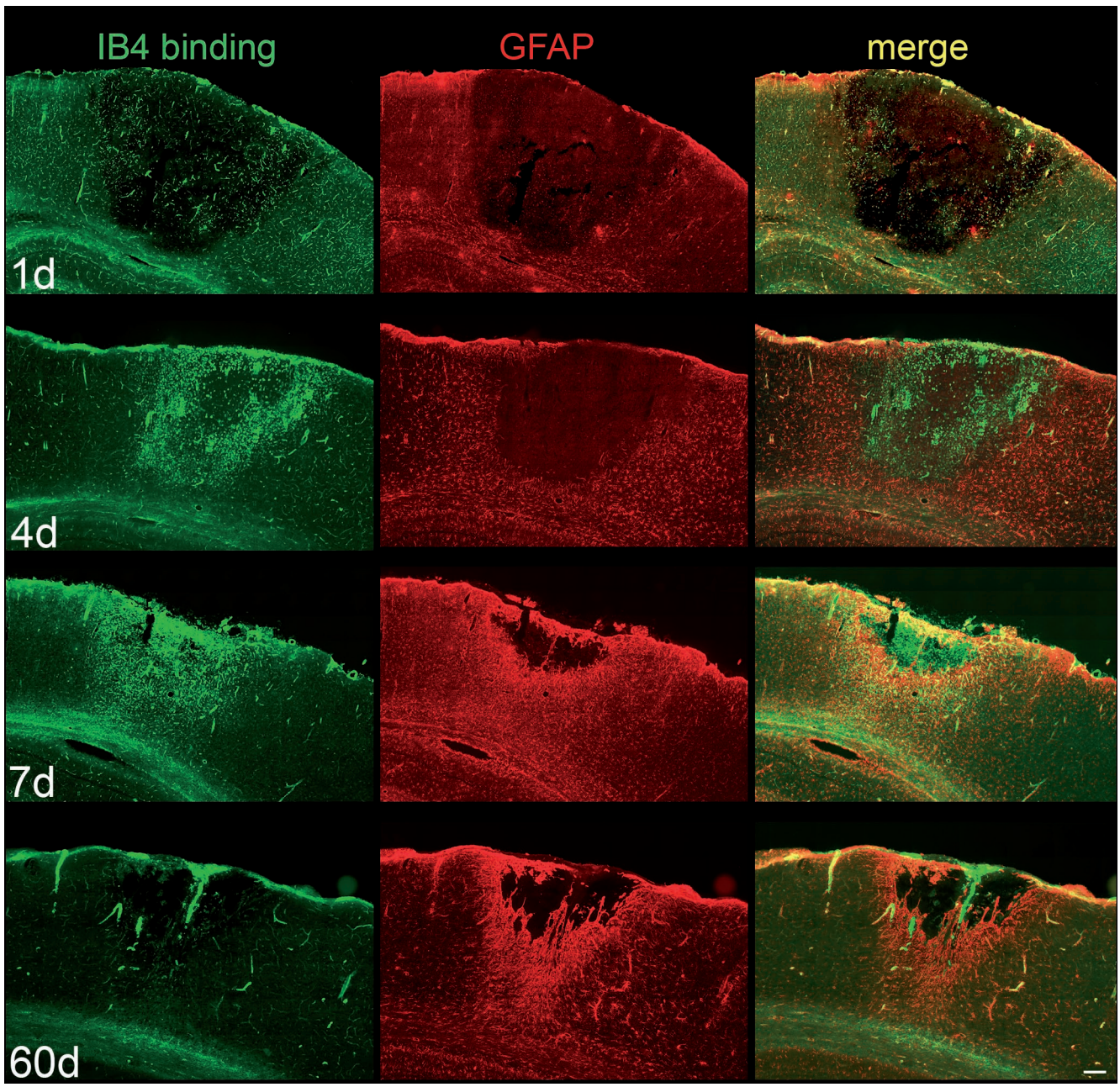

Fig. 7. Comparison of astroglial and microglial response at the infarct site 1, 4, 7, and 60 days after photothrombosis as revealed by double immunofluorescence. Note that 4 days post lesion GFAP (red) is spatially separated from IB4 staining (green). GFAP was observed outside the lesion whereas microglia were concentrated inside the infarct, near its border. Seven days after stroke activated microglia invade the area occupied by reactive astrocytes. At day 60 no reactive microglia could be observed around the lesion. Scale bar is 200 μm.

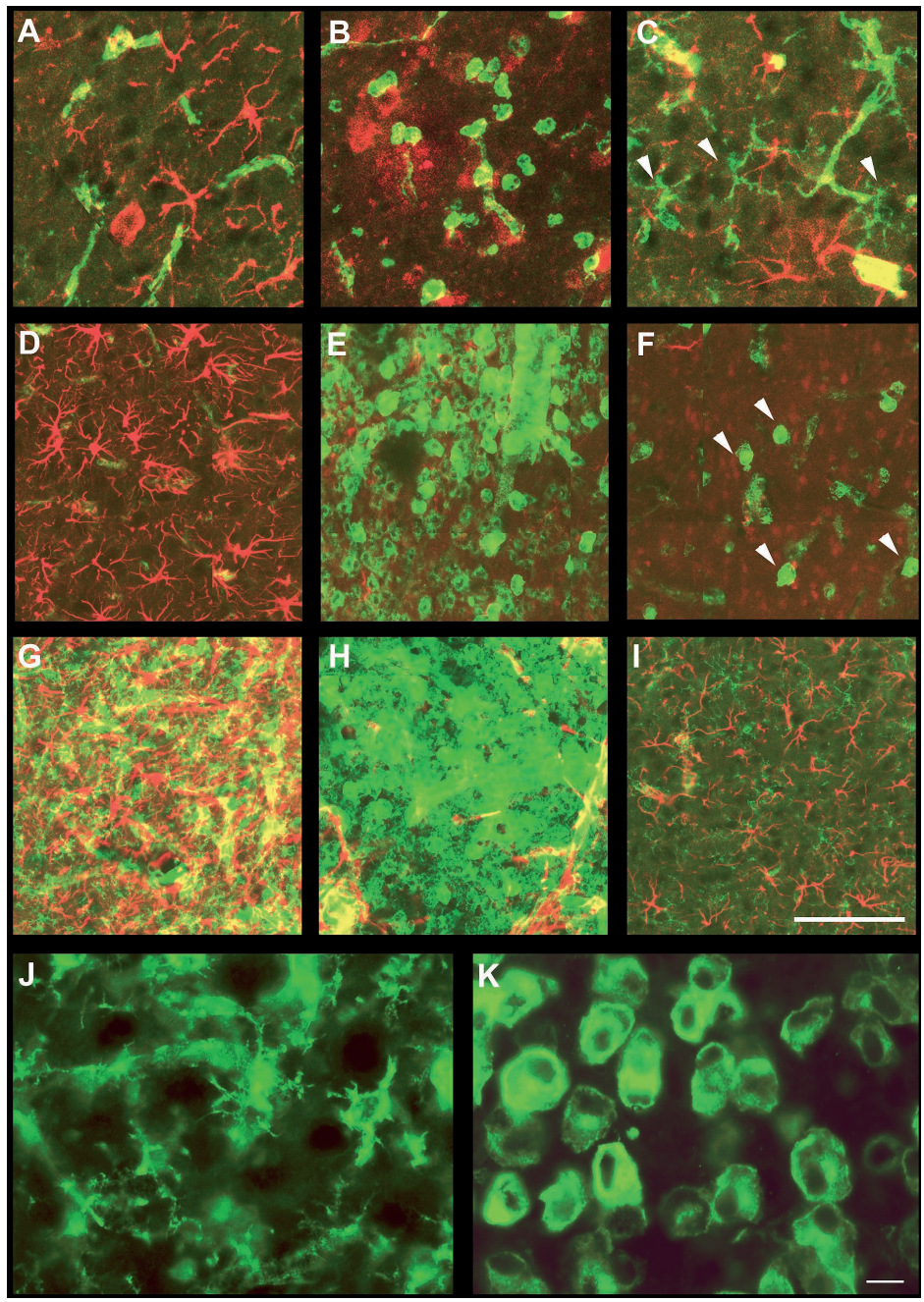

Fig. 8. Double immunofluorescence for astrocytes stained for GFAP and microglia stained with IB_4 1 (A–C), 4 (D–F), and 7 (G–K) days after photothrombosis. (A) shows staining in area close to the infarct. Note the lack of IB_4 – positive microglial cells. Green immunofluorescence refers to non-specific staining of blood vessels. IB_4-positive ameboid cells were present in the infarct core, but close to its boundary (B). In areas remote to the lesion numerous ramified microglial cells could be found [arrowheads in (C)]. (D) shows reactive astrocytes close to the lesion border. Four days after the lesion still no IB4-positive microglia could be seen in this area. (E) shows the area of the lesion contiguous to non-lesioned tissue, with numerous ameboid microglial cells. In the very center of the lesion only few such cells could be found [arrowheads in (F)]. Seven days after lesion in cortical tissue surrounding the infarct both activated astrocytes and microglia could be observed (G). The core of the infarct was filled with ameboid cells (H). In remote cortical areas resting ramified microglia and numerous fibrous astrocytes were present (I). (J, K), high power microphotographs showing the details of the structure of microglia from the perilesional area (J), and infarct core (K). Large scale bar is 100 μm (A–I); small scale bar is 10 μm (J–K).

leagues (1995) suggest that remote astrogliosis is triggered by the episodes of transient suppression of cortical activity, propagating in a wavelike fashion, called spreading depression (SD), occurring in response to ischemic insult (Dietrich et al. 1994). This idea is supported by the fact that blocking NMDA receptors with MK-801 suppresses SD and abolishes the increase in GFAP expression in the lesioned hemisphere (Schroeter et al. 1995, Yamashita et al. 1996).

We found with the lectin histochemistry, that activated microglial cells were abundant in the vicinity of the infarct as soon as 24 hours after stroke. Because it is not possible to distinguish between resident and blood derived macrophages we were not able to identify the source of this ameboid IB_4-positive cells. It seemed however, that at least part of this population was of microglial origin, because the neighboring areas of the cortex were strikingly "empty" during first few days after stroke as if all microglial cells migrated into boundary of the lesion. Moreover, studies in transgenic chimeric mice with GFP-expressing bone marrow-derived cells showed that infiltrating macrophages were observed in later stages of lesion development and the vast majority of macrophages in the infarct area were of local origin (Schilling et al. 2003, Denker et al. 2007). No microglial reaction was observed in remote regions both in lesioned and contralateral hemispheres. These results are in contrast to the changes reported after middle cerebral artery occlusion by Morioka and coauthors (1993), who found that the contralateral cortex and hippocampus showed the presence of activated microglia. The reasonable explanation of these discrepancies is the difference of the method used. MCAO leads to large infarctions including subcortical areas, thus producing many secondary effects and a very strong glial response whereas photothrombotic lesion is usually confined to the cortex. Neither our data confirmed earlier reports by Morioka and others (1993) and Schroeter and coauthors (1999) that after photothrombotic lesion activation of microglia occurred in subcortical areas at the level of infarct. At all examined levels we did not observe activation of microglia as revealed by isolectin B_4 staining. We would attribute these dissimilarities to small but important differences in experimental conditions, resulting in larger infarcts affecting strongly subcortical white matter (e.g. Fig 3 in Schroeter et al. 2002).

CONCLUSIONS

Our data demonstrated a widespread astrocytic response in non-ischemic areas of the brain subjected to unilateral photothrombotic lesion, in contrast to strictly defined microglial reaction, limited to the perilesional area. The nature of this astrocytic response is still elusive and requires extensive investigation. On the one hand, astrogliosis may contribute to damage by propagation of SD waves and by excreting detrimental substances. On the other hand, reactive astrocytes are thought to produce potentially neuroprotective growth factors and cytokines. Thus, the precise characterization of the extent of astrocytic response to stroke appears to be of a great importance.

ACKNOWLEDGEMENTS

This study was supported by Polish-German MSRI grant K 057/P05/2003 and by statutory funds of the Nencki Institute.

REFERENCES

Allen GV, Cheung RT, Cechetto DF (1995) Neurochemical changes following occlusion of the middle cerebral artery in rats. Neuroscience 68: 1037–1050.

Anderson MF, Blomstrand F, Blomstrand C, Eriksson PS, Nilsson M (2003) Astrocytes and stroke: networking for survival? Neurochem Res 28: 293–305.

Bidmon HJ, Jancsik V, Schleicher A, Hagemann G, Witte OW, Woodhams P, Zilles K (1998) Structural alterations and changes in cytoskeletal proteins and proteoglycans after focal cortical ischemia. Neuroscience 82: 397–420.

Bignami A, Dahl D (1977) Specificity of the glial fibrillary acidic protein for astroglia. J Histochem Cytochem 25: 466–469.

Cheung RT, Diab T, Cechetto DF (1995) Time-course of neuropeptide changes in peri-ischemic zone and amygdala following focal ischemia in rats. J Comp Neurol 360: 101–120.

Davies CA, Loddick SA, Stroemer RP, Hunt J, Rothwell NJ (1998) An integrated analysis of the progression of cell responses induced by permanent focal middle cerebral artery occlusion in the rat. Exp Neurol 154: 199–212.

Denker SP, Ji S, Dingman A, Lee SY, Derugin N, Wendland MF, Vexler ZS (2007) Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. J Neurochem 100: 893–904.

Dietrich WD, Feng ZC, Leistra H, Watson BD, Rosenthal M (1994) Photothrombotic infarction triggers multiple episodes of cortical spreading depression in distant brain regions. J Cereb Blood Flow Metab 14: 20–28.

Durukan A, Tatlisumak T (2007) Acute ischemic stroke: Overview of major experimental models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav 87: 179–197.

Fawcett JW, Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull 49: 377–391.

Figueiredo BC, Skup M, Bedard AM, Tetzlaff W, Cuello AC (1995) Differential expression of p140trk, p75NGFR and growth-associated phosphoprotein-43 genes in nucleus basalis magnocellularis, thalamus and adjacent cortex following neocortical infarction and nerve growth factor treatment. Neuroscience 68: 29–45.

Forsyth RJ (1996) Astrocytes and the delivery of glucose from plasma to neurons. Neurochem Int 28: 231–241.

Giulian D, Vaca K, Corpuz M (1993a) Brain glia release factors with opposing actions upon neuronal survival. J Neurosci 13: 29–37.

Giulian D, Corpuz M, Chapman S, Mansouri M, Robertson C (1993b) Reactive mononuclear phagocytes release neurotoxins after ischemic and traumatic injury to the central nervous system. J Neurosci Res 36: 681–693.

Gregersen R, Lambertsen K, Finsen B (2000) Microglia and macrophages are the major source of tumor necrosis factor in permanent middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 20: 53–65.

Haupt C, Witte OW, Frahm C (2007) Temporal profile of connexin 43 expression after photothrombotic lesion in rat brain. Neuroscience 144: 562–570.

Herrera DG, Cuello AC (1992) Glial fibrillary acidic protein immunoreactivity following cortical devascularizing lesion. Neuroscience 49: 781–791.

Jablonka J, Kossut M (2006) Focal stroke in the barrel cortex of rats enhances ipsilateral response to vibrissal input. Acta Neurobiol Exp (Wars) 66: 261–266.

Kirchhoff F, Dringen R, Giaume C (2001) Pathways of neuron-astrocyte interactions and their possible role in neuroprotection. Eur Arch Psychiatry Clin Neurosci 251: 159–169.

Ludwin SK, Kosek JC, Eng LF (1976) The topographical distribution of S-100 and GFA proteins in the adult rat brain: an immunohistochemical study using horseradish peroxidase-labeled antibodies. J Comp Neurol 165: 197–207.

McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Prog Neurobiol 55: 257–332.

McGraw J, Hiebert GW, Steeves JD (2001) Modulating astrogliosis after neurotrauma. J Neurosci Res 63: 109–115.

Monzon-Mayor M, Alvarez M, Arbelo-Galvan J, Romero-Aleman M, Yanes C, Plaza ML, Rodriguez JR, Rodriguez JJ, Toledano A (2000) Long-term evolution of local, proximal and remote astrocyte responses after diverse nucleus basalis lesioning (an experimental Alzheimer model): GFAP immunocytochemical study. Brain Res 865: 245–258.

Morioka T, Kalehua AN, Streit WJ (1993) Characterization of microglial reaction after middle cerebral artery occlusion in rat brain. J Comp Neurol 327: 123–132.

Nakata N, Kato H, Kogure K (1993) Protective effects of basic fibroblast growth factor against hippocampal neuronal damage following cerebral ischemia in the gerbil. Brain Res 605: 354–356.

Olson EE, McKeon RJ (2004) Characterization of cellular and neurological damage following unilateral hypoxia/ischemia. J Neurol Sci 227: 7–19.

Paxinos G, Watson C (2005) Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA.

Raivich G, Jones LL, Werner A, Bluthmann H, Doetschmann T, Kreutzberg GW (1999) Molecular signals for glial activation: pro- and anti-inflammatory cytokines in the injured brain. Acta Neurochir Suppl 73: 21–30.

Rogozinska K, Skangiel-Kramska J (2005) D1 dopamine receptor distribution following photothrombotic stroke in rat cerebral cortex. Acta Neurobiol Exp (Wars) 65: 167–172.

Rojas S, Martín A, Justicia C, Falcón C, Bargalló N, Chamorro A, Planas AM (2006) Modest MRI signal intensity changes precede delayed cortical necrosis after transient focal ischemia in the rat. Stroke 37: 1525–1532.

Savchenko VL, McKanna JA, Nikonenko IR, Skibo GG (2000) Microglia and astrocytes in the adult rat brain: comparative immunocytochemical analysis demonstrates the efficacy of lipocortin 1 immunoreactivity. Neuroscience 96: 195–203.

Schilling M, Besselmann M, Leonhard C, Mueller M, Ringelstein EB, Kiefer R (2003) Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. Exp Neurol 183: 25–33.

Schroeter M, Schiene K, Kraemer M, Hagemann G, Weigel H, Eysel UT, Witte OW, Stoll G (1995) Astroglial responses in photochemically induced focal ischemia of the rat cortex. Exp Brain Res 106: 1–6.

Schroeter M, Jander S, Witte OW, Stoll G (1999) Heterogeneity of the microglial response in photochemically induced focal ischemia of the rat cerebral cortex. Neuroscience 89: 1367–1377

Schroeter M, Franke C, Stoll G, Hoehn M (2001) Dynamic changes of magnetic resonance imaging abnormalities in relation to inflammation and glial responses after photothrombotic stroke cerebral infarction in the rat brain. Acta Neuropathol 101: 114–122.

Schroeter M, Jander S, Stoll G (2002) Non-invasive induction of focal cerebral ischemia in mice by photothrombosis of cortical microvessels: characterization of inflammatory responses. J Neurosci Methods 117: 43–49.

Shanina EV, Redecker C, Reinecke S, Schallert T, Witte OW (2005) Long-term effects of sequential cortical infarcts on scar size, brain volume and cognitive function. Behav Brain Res 158: 69–77.

Song H, Stevens CF, Gage FH (2002) Astroglia induce neurogenesis from adult neural stem cells. Nature 417: 39–44.

Stoll G, Jander S, Schroeter M (1998) Inflammation and glial responses in ischemic brain lesions. Prog Neurobiol 56: 149–171.

Streit WJ, Kreutzberg GW (1987) Lectin binding by resting and reactive microglia. J Neurocytol 16: 249–260.

Sulejczak D, Czarkowska-Bauch J, Macias M, Skup M (2004) Bcl-2 and Bax proteins are increased in neocortical but not in thalamic apoptosis following devascularizing lesion of the cerebral cortex in the rat: an immunohistochemical study. Brain Res 1006: 133–149.

Wang K, Walz W (2003) Unusual topographical pattern of proximal astrogliosis around a cortical devascularizing lesion. J Neurosci Res 73: 497–506.

Watson BD, Dietrich WD, Busto R, Wachtel MS, Ginsberg MD (1985) Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17: 497–504.

Witte OW, Bidmon HJ, Schiene K, Redecker C, Hagemann G (2000) Functional differentiation of multiple perilesional zones after focal cerebral ischemia. J Cereb Blood Flow Metab 20: 1149–1165.

Wong ML, Loddick SA, Bongiorno PB, Gold PW, Rothwell NJ, Licinio J (1995) Focal cerebral ischemia induces CRH mRNA in rat cerebral cortex and amygdala. Neuroreport 6: 1785–1788.

Yamashita K, Vogel P, Fritze K, Back T, Hossmann KA, Wiessner C (1996) Monitoring the temporal and spatial activation pattern of astrocytes in focal cerebral ischemia using in situ hybridization to GFAP mRNA: comparison with sgp-2 and hsp70 mRNA and the effect of glutamate receptor antagonists. Brain Res 735: 285–297.

Yao C, Williams AJ, Hartings JA, Lu XC, Tortella FC, Dave JR (2005) Down-regulation of the sodium channel Na(v)1.1 alpha-subunit following focal ischemic brain injury in rats: in situ hybridization and immunohistochemical analysis. Life Sci 77: 1116–1129.