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Expression of 27 kDa Heat Shock Protein (Hsp27) in Immature Rat Brain After a Cortical Aspiration Lesion

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KEY WORDS astrocytes; brain injury; Hsp27; microglia; postnatal development; stress response; vimentin

ABSTRACT The 27 kDa heat shock protein (Hsp27) is a well-known member of the astroglial response to injury, playing a protective role against oxidative stress, apoptosis, and cytoskeletal destruction. Although several studies have been focused on the damaged adult brain, little is known about Hsp27 expression in the immature brain. In this work, we have examined the spatiotemporal pattern of Hsp27 expression in the normal postnatal rat brain following a cortical aspiration lesion at postnatal day 9. In the immature brain, Hsp27 is mainly observed in the internal capsule, although some scattered cells are also found in the ependyma, the corpus callosum, the septum, and hypothalamic glia limitans. In the internal capsule, Hsp27 expression is developmentally regulated, being significantly decreased from postnatal day 14. After a cortical aspiration lesion, de novo expression of Hsp27 is observed in cortical injured areas as well as in the secondary affected thalamus. In the cortex, expression of Hsp27 is first seen at day 1 postlesion (PL) surrounding the neurodegenerative area, becoming restricted to the glial scar at longer survival times. Although a pulse-like expression of Hsp27 is observed in some microglial cells at day 1 PL, most Hsp27-labeled cells are reactive astrocytes, which show GFAP overexpression and coexpress vimentin from day 3 PL. In the thalamus, astroglial Hsp27 expression is delayed, being first observed at day 5 PL. Thalamic Hsp27-labeled astrocytes do not show vimentin expression. Our observations demonstrate astroglial expression of Hsp27 in areas of tissue damage following postnatal traumatic injury, suggesting an involvement of this cytoskeleton-stabilizing protein in the remodeling processes following postnatal brain damage. *GLIA 36: 259–270, 2001.*

INTRODUCTION

Heat shock protein 27 (Hsp27) is a member of the family of small heat shock proteins (sHsp) that has been involved in several protective mechanisms. In vitro studies have demonstrated that Hsp27 acts as a molecular chaperone (Ehrnsperger et al., 1998) and as an actin capping-decapping protein stabilizing the cytoskeleton (Lavoie et al., 1993). In addition, Hsp27 seems to provide neuroprotection against TNF-α–induced oxidative stress by abolishing the burst of intracellular reactive oxygen species (ROS) (Mehlen et al., 1996a) and against Fas/APO-1 and staurosporine-induced apoptotic cell death (Mehlen et al., 1996b).

In the adult central nervous system (CNS), Hsp27 is constitutively expressed in brainstem neurons (Costigan et al., 1998; Hopkins et al., 1998) and in some

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white matter astroglial cells (Plumier et al., 1996), increasing its expression after brain damage. Brain stem neurons upregulate Hsp27 synthesis in response to axotomy (Costigan et al., 1998; Hopkins et al., 1998), whereas astrocytes and, to a lesser extent, microglial cells show de novo expression of Hsp27 following different types of experimentally induced lesions, including photothrombotic injury (Plumier et al., 1997a), focal cerebral ischemia (Kato et al., 1995), seizures (Plumier et al., 1996), and spreading depression (Plumier et al., 1997b). Furthermore, Hsp27 expression is also upregulated in glial cells in neurodegenerative conditions such as Alzheimer's disease (Renkawek et al., 1994).

Other members of the family of heat shock proteins, such as Hsc70, Hsp90, Hsp60, or Hsp32, have been shown to be transiently expressed during embryonic and postnatal development (Bergeron et al., 1998; Brown and Gozes, 1998; Brown and Sharp, 1999; Morange, 1999). Accordingly, Hsp27 expression is also developmentally regulated (Arrigo, 1995). In the embryonic and early postnatal life, Hsp27 accumulates during neuroectodermal cell differentiation (Walsh et al., 1997) and in developing spinal cord neurons, Purkinje cells (Gernold et al., 1993), and dorsal root ganglia (Costigan et al., 1998; Lewis et al., 1999), where Hsp27 has been implicated in the regulation of cell differentiation (Arrigo, 1995) and the protection against environmental or physiological stress (Arrigo, 1995; Costigan et al., 1998; Lewis et al., 1999). No studies, however, are available on glial Hsp27 expression in the postnatal rat brain, a period characterized by exceptional plasticity and neuroprotective mechanisms, reorganization of synaptic connectivity, high amounts of neurotrophic factors, and differentiation of glial cells (Kolb et al., 1994, 1996; Snyder et al., 1998; Werther et al., 1998). As postnatal astrocytes seem to contribute to these events by playing a neuronotrophic role during development (Snyder et al., 1998; Werther et al., 1998) and Hsp27 is recognized as a component of the astroglial stress response (Plumier et al., 1996, 1997a, 1997b), the aim of the present work was to elucidate the possible involvement of Hsp27 in the glial response to traumatic injury in the immature rat brain.

MATERIALS AND METHODS

Aspiration Lesion

Long Evans black-hooded 9-day-old rats (P9; Iffa-Credo, Lyon, France) of both sexes were used in this study. Under ether anesthesia, each rat was placed in a stereotactic frame adapted for newborns (Kopf), and the skull was opened using a trephine. The forelimb (FL) area of the sensorimotor cortex (2 mm lateral from bregma) was removed using a Pasteur pipette (1 mm diameter) connected to a vacuum pump, removing a cylinder of tissue of approximately 1.2 mm^3. After suture, rats were placed in an incubator and maintained at 36°C for 2 h before being returned to their mothers. Four lesioned animals, two sham-operated and two control animals, were used for each postlesion (PL) survival times. This experimental procedure was approved by the Ethical Commission of the Autonomous University of Barcelona. All efforts were made to minimize animal suffering in every step.

Fixation and Histology

At 4 and 10 h and 1, 3, 5, and 7 days PL, animals were anesthetized by ether inhalation and perfused intracardially for 10 min with 4% paraformaldehyde in 0.1 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.4). Brains were removed and kept in the same fixative for 4 h, cryoprotected in a 30% sucrose solution in 0.1 M phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.4), and frozen with dry CO_2. Frozen coronal sections (30 μm) were obtained and stored free-floating in De Olmos antifreeze solution (Warr et al., 1981). Alternate cryostat sections (20 μm) were mounted on gelatin-coated slides and stained with toluidine blue for routine histological examination. Free-floating cryostat sections were processed for immunohistochemistry and/or histochemistry.

Immunohistochemistry for Hsp27, Glial Fibrillary Acidic Protein (GFAP), and Vimentin

After blocking endogenous peroxidase with 2% H_2O_2, 70% methanol in 0.05 M Tris buffered saline (TBS; pH 7.4), cryostat sections were treated with 10% fetal calf serum (FCS) in TBS containing 1% Triton X-100 (TBS-T; pH 7.4) for 30 min and incubated overnight at 4°C with primary antibody in TBS-T containing 10% FCS. Either one of the following were used as primary antibodies: rabbit anti-Hsp25 antibody (StressGen, SPA-801, U.S.; 1:2,500; Hsp27 detection), rabbit anti-GFAP antibody (Dako, Z-0334, Denmark; 1:1,800) or mouse anti-vimentin (Dako, M-725; 1:1,000). After washing with TBS-T, the sections were incubated at room temperature for 1 h with the secondary biotinylated antibody, an anti-rabbit donkey antibody (Amersham, RPN1004, U.K.; 1:200) for Hsp27 and GFAP and a anti-mouse sheep antibody (Amersham, RPN1001; 1:200). Sections were rinsed in TBS-T and incubated for 1 h at room temperature with avidin-peroxidase (Dako, P364; 1:400) in 10% FCS in TBS. After rinsing again in TBS-T, the peroxidase reaction product was visualized by incubating the sections in 100 ml of 0.05 M Tris buffer (pH 7.4) containing 50 mg 3,3′-diaminobenzidine (DAB) and 33 μl of hydrogen peroxide (30%). As negative controls, sections were incubated in media lacking primary antibodies. Finally, sections were mounted on gelatin-coated slides, dehydrated in increasing concentrations of ethanol, cleared in xylene, and coverslipped in DPX mountant medium (Bancroft and Stevens, 1996).

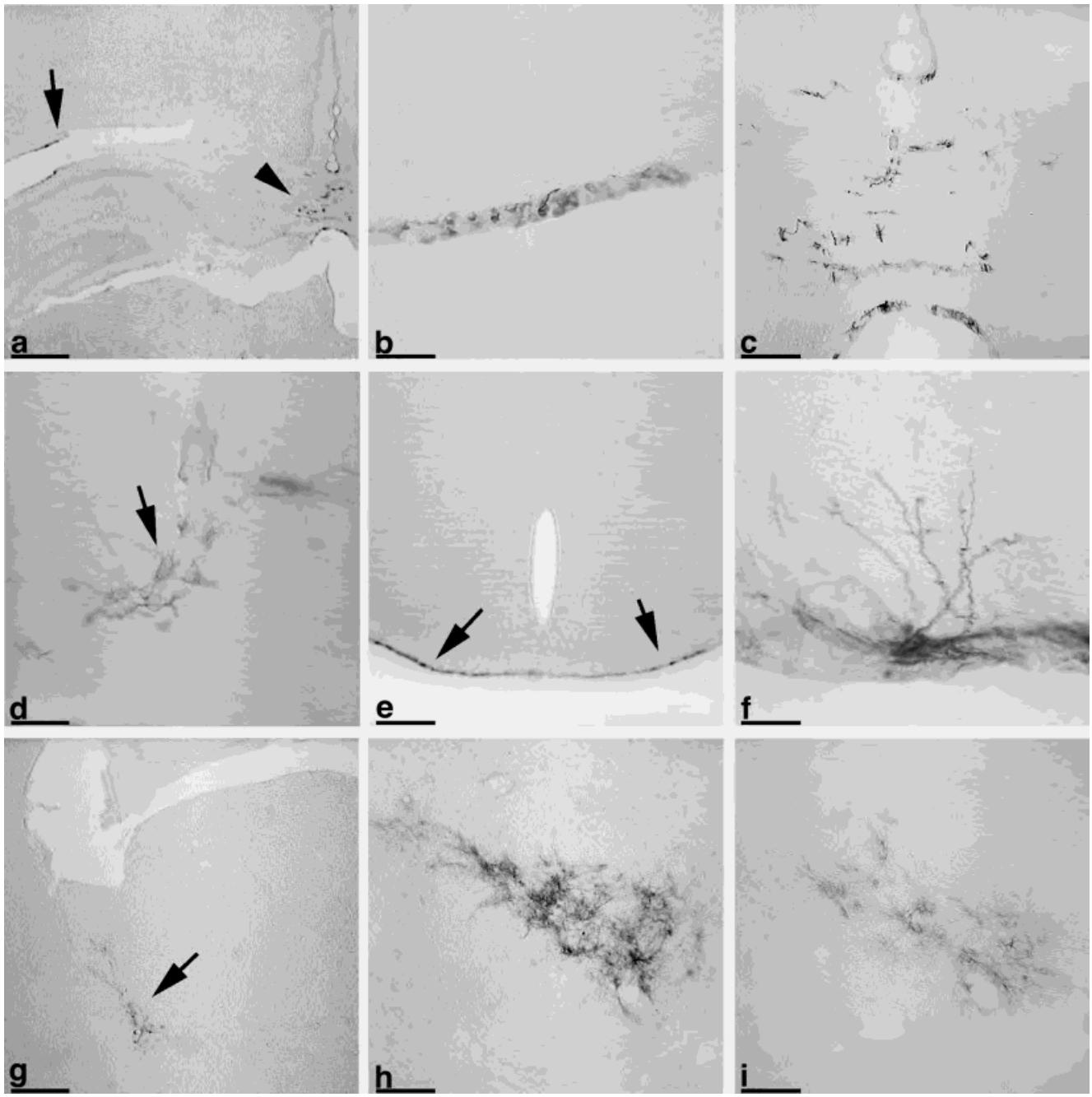

Fig. 1. Micrographs showing the main areas of Hsp27 expression in control postnatal animals. Hsp27 expression is observed in the corpus callosum (arrowheads in **a**, **c**, and **d**), where Hsp27 immunoreactive cells are often seen in close association with blood vessels (arrows in **d**). Other Hsp27 expressing areas include ependymal cells of lateral ventricles (b) and the hypothalamic glia limitans (**e** and **f**). However, the main area showing Hsp27 expression is the internal capsule (arrows in **g**–**i**). In this white matter tract, Hsp27 expression decreased with age: panel e shows postnatal day 9 and panel f shows postnatal day 16. Scale bars: a, e, and g, 355 μm; b and d, 35 μm; c, h, and i, 90 μm.

Tomato Lectin Histochemistry

Tomato lectin histochemistry was used for the specific visualization of microglial cells as previously described (Acarin et al., 1994). Briefly, after endogenous peroxidase blocking with 2% H_2O_2, 70% methanol in TBS, free-floating sections were rinsed in TBS (pH 7.4) containing 0.5% Triton X-100 and incubated for 2 h at room temperature with the biotinylated tomato (*Lycopersicon esculentum*) lectin (Sigma L-9389, St. Louis, MO) diluted to 6 μg/ml in TBS plus 0.5% Triton X-100. After incubation, sections were washed in TBS and incubated for 1 h at room temperature with avidin peroxidase (Dako, P364) in a 1:400 dilution in TBS. Following several rinses in TBS, the peroxidase reaction product was visualized using 50 mg of 3,3′-diami-

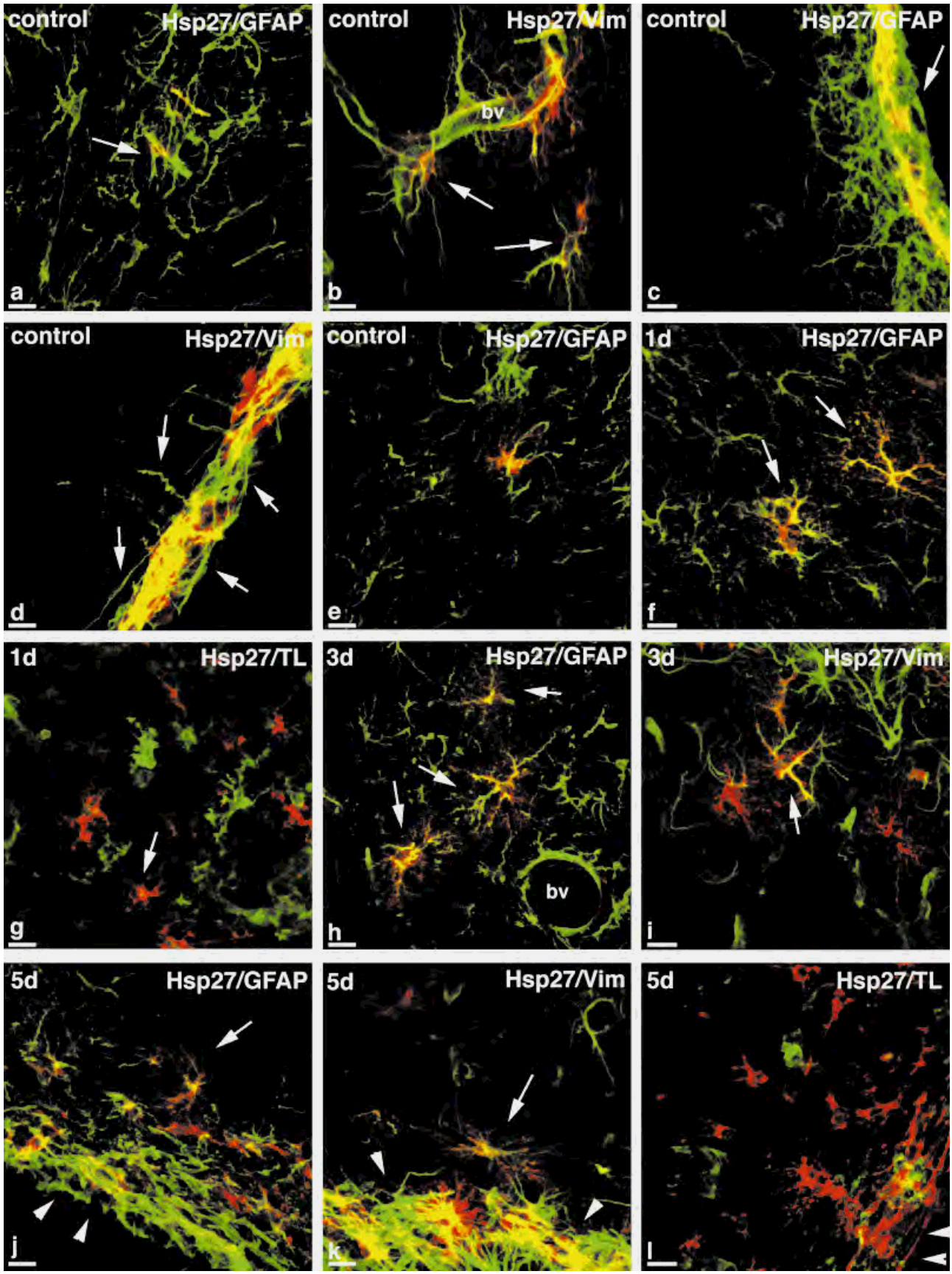

Fig. 2. Identification of Hsp27-positive cells in the postnatal control (**a**–**e**) and injured (**f**–**l**) rat brain. Areas shown include the corpus callosum (a and b), glia limitans of the hypothalamus (c and d), and internal capsule (e) in the control brain and the lesioned cortex at day 1 (f and g) and day 3 (h and i) and day 5 (j–i) PL. Hsp27 is shown in red; GFAP, vimentin (Vim) and tomato lectin (TL) are shown in green, and yellow areas show colocalization (arrows point to double-labeled cells in all panels). In control brains, Hsp27-labeled cells of the corpus callosum and glial limitans show GFAP (a and c) and vimentin (b and d) immunoreactivity. In the injured cortex, at 1 day PL, Hsp27-labeled cells are identified as astrocytes (f), although some microglial cells are also positive (g). At longer survival times, Hsp27 is exclusively found in reactive astrocytes (h, j, and l) coexpressing vimentin (i, k). Arrowheads in j, k, and l show the glial scar. Scale bars, 10 μm.

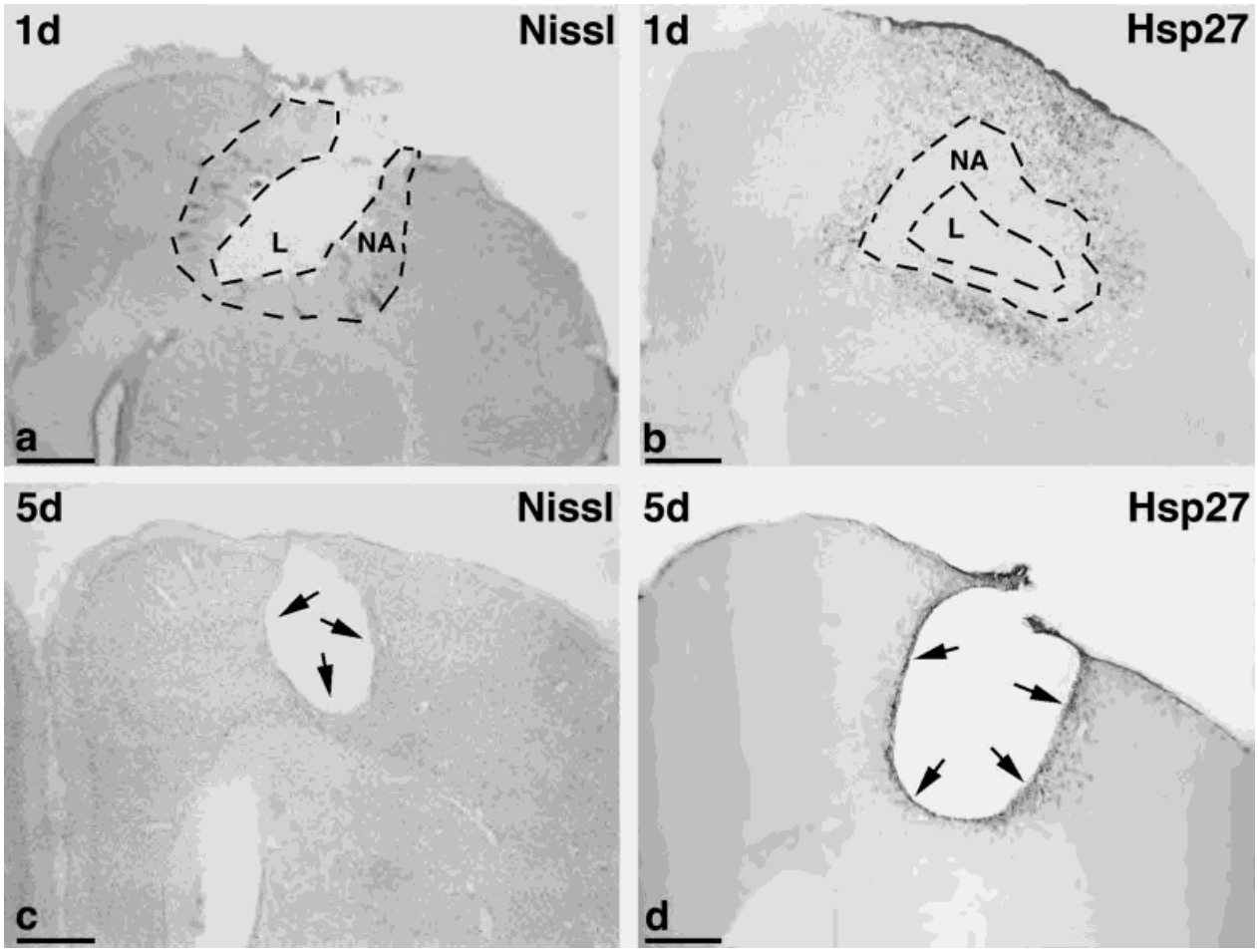

Fig. 3. Illustrations showing histopathological characteristics (**a** and **c**) and Hsp27 distribution (**b** and **d**) in the injured cortex at day 1 PL (a and b) and day 5 PL (c and d). At day 1 PL (a and b), the neurodegenerative area (NA; delimited by broken lines) is found surrounding the lesion cavity (L; a), whereas Hsp27 is observed in the tissue surrounding the NA (b). At later time points (c and d), Hsp27 is exclusively observed at the glial scar (arrows in c and d). Scale bars, 500 μm.

nobenzidine (DAB) with 33 μl of hydrogen peroxide (30%) in 100 ml 0.05 M Tris buffer (pH 7.4). Control of tomato lectin specificity was carried out by preincubating some sections for 30 min in a 0.1 M solution of N-acetyl-lactosamine (Sigma, A-7791).

Double Fluorescence Immunohistochemistry

For the simultaneous visualization of Hsp27 and glial cells (GFAP and vimentin for astrocytes and tomato lectin for microglia), some free-floating sections were immunostained for Hsp27 as described above, but using Cy3-conjugated anti-rabbit IgG (Amersham, PA-43004; 1:1,000) as secondary antibody. Double labeling for either GFAP, vimentin, or tomato lectin was visualized as reported but using a Cy2-conjugated avidin (Amersham, PA-42000; 1:1,000). Double-stained sections were analyzed using a LEICA TCS 4D confocal microscope.

RESULTS

Constitutive Hsp27 Expression in Developing Brain

In control and sham-operated animals, constitutive expression of Hsp27 was exclusively found in relation to glial cells; no immunoreactivity was observed in neurons or blood vessel endothelial cells. Scattered Hsp27-labeled glial cells were found in the ependyma of lateral ventricles (Fig. 1a and b), the corpus callosum (Fig. 1a, c, and d), the septum, and forming the glia limitans of the hypothalamus (Fig. 1e and f), although most Hsp27-positive cells were observed in the internal capsule (Fig. 1g-i), where expression was developmentally regulated. Hsp27 expression did not apparently change between postnatal day 9 (P9) and P12 (Fig. 1h), but it became significantly decreased from P14 (Fig. 1i).

According to double labeling with antibodies against GFAP (Fig. 2a, c, and e), Hsp27 positive cells were identified as astrocytes. Noteworthy is that HSP27-positive cells represented only a subpopulation of as-

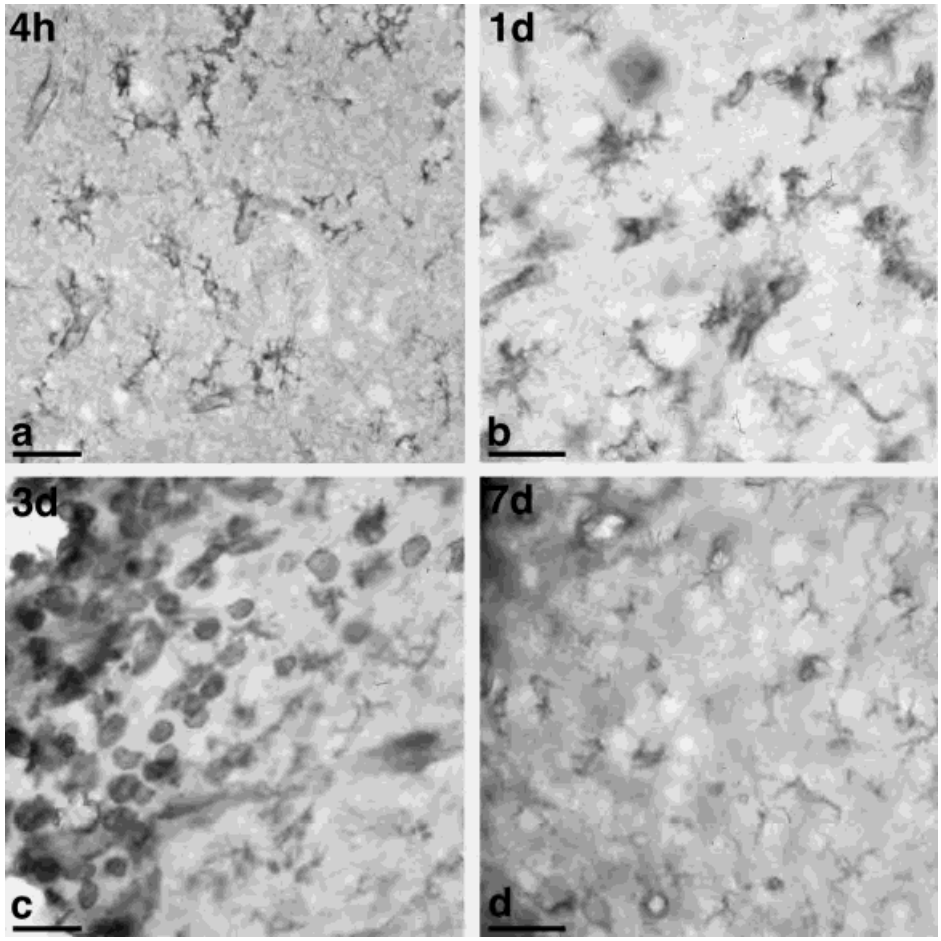

Fig. 4. Micrographs showing the microglial reactivity in the injured cortex by means of tomato lectin histochemistry, at 4 h (**a**), day 1 PL (**b**), day 3 PL (**c**), and day 7 PL (**d**). Mildly reactive microglial cells are observed at 4 h PL (a), whereas cells become pseudopodic (b) and round (c) at days 1 and 3 PL, respectively. At 7 days PL, the microglial response is clearly diminished. Scale bars, 25 μm.

troglial cells. In addition, Hsp27 immunoreactive cells from the corpus callosum (Fig. 2b), hypothalamus (Fig. 2d), septum, and ependyma also coexpressed the cytoskeletal protein vimentin. Hsp27-labeled astrocytes displayed a highly ramified morphology and their processes were often found in close association with blood vessels (Fig. 1d and 2b). The astroglial cell body and major processes were highly immunoreactive for Hsp27, GFAP, and vimentin, whereas minor processes showed milder Hsp27 staining and absence of GFAP or vimentin.

Hsp27 Expression and Glial Response in Injured Postnatal Brain

Histopathology

The cortical aspiration lesion generated a cavity surrounded by a neurodegenerative area (NA), which until day 1 PL appeared as a light band 500–700 μm wide occupied by degenerating neurons and blood infiltrates (Fig. 3a). The NA was progressively reduced from day 3 PL, becoming the glial scar at 5 days PL (Fig. 3c). As early as 4 h PL, the tissue surrounding the NA had lost its characteristic cortical laminated pattern, which was not recovered until day 5 PL. In addition to cortical degeneration, a distal area of secondary degeneration was observed in the inferior third of the thalamic ventrobasal complex (VB), where neuronal loss was evident at day 7 PL.

Glial reactivity

A strong astrocytic and microglial reactivity was observed both in the lesioned cortex and in the ipsilateral thalamus. In the cortex, microglial reactivity was characterized by a rapid onset, increasing the amount of amoeboid and reactive ramified microglial morphologies in the NA and the surrounding tissue as early as 4 h PL (Fig. 4a). Cortical microglial reactivity peaked between days 1–3 PL (Fig. 4b and c), as assessed by the strong increase in lectin binding, and returned to resting morphologies and basal lectin binding by day 7 PL (Fig. 4d). Moreover, in the tissue surrounding the NA, hypertrophied astrocytes expressing increased levels of GFAP were observed from 10 h PL (Fig. 5a). Some of these reactive astrocytes also expressed vimentin from 3 days PL and were mainly found in the borders of the lesion cavity (Fig. 5d and f).

In comparison to cortical areas, the ipsilateral VB thalamic complex and the centrolateral (CL) nucleus

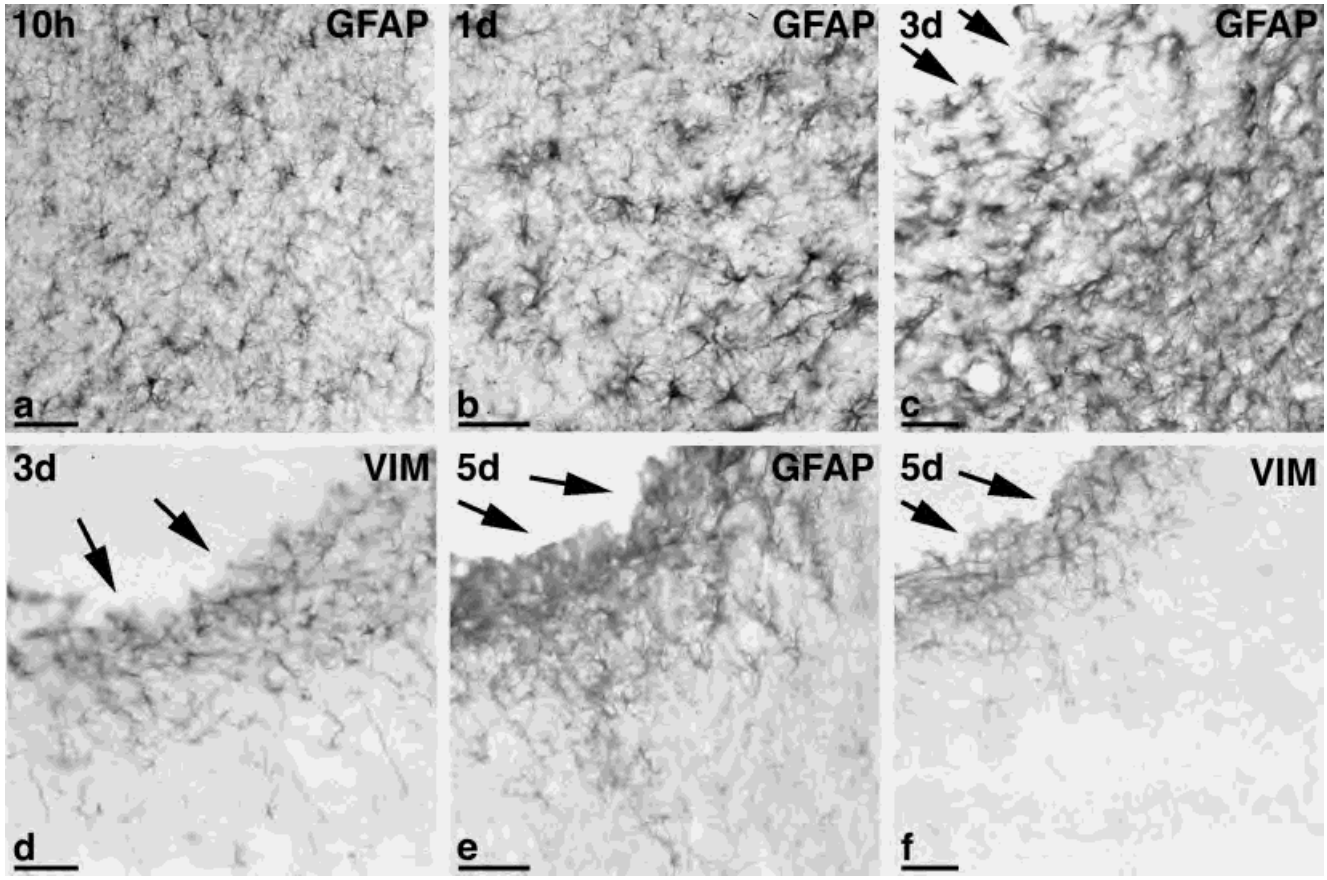

Fig. 5. Micrographs showing the astroglial reactivity in the injured cortex by means of GFAP (**a–c** and **e**) and vimentin (VIM; **d** and **f**) immunohistochemistry, at 10 h (a), day 1 (b), day 3 (c and d), and day 5 (e and f) PL. GFAP overexpression is already observed at 10 h PL (a) and astroglial cells become further hypertrophied at longer survival times (b, c, and e), when they also express vimentin (d and f). Arrows in plates c–f show the borders of the cavity. Scale bars, 35 μm.

showed a slightly protracted pattern of glial reactivity. Reactive microglial cells, displaying ameboid and reactive ramified morphologies, were observed from 10 h PL (Fig. 6a) and reached maximal response at day 3 PL (Fig. 6d and e). Thalamic astroglial reactivity was even more delayed, being first noticed at day 1 PL (Fig. 7a) and peaking between days 3 and 5 PL (Fig. 7b and c). Noteworthy is that thalamic reactive astrocytes did not express vimentin at any of the survival times studied.

Induction of Hsp27 expression

Expression of Hsp27 in cortical areas was not noticed until day 1 after the aspiration lesion (Fig. 8a), when Hsp27 was observed in a thin tissue band of 1 mm width surrounding the NA (Fig. 3b). Hsp27-positive cells showed a highly ramified morphology and were mainly identified as astrocytes by their double labeling with GFAP (Fig. 2f). These reactive astroglial cells showed colocalization of Hsp27 and GFAP in the cell body and major processes, whereas minor processes were Hsp27-positive but apparently lacked GFAP. Later on, at days 3 and 5 PL, Hsp27-labeled astrocytes formed a dense cellular network surrounding the lesion cavity (Fig. 8b and c), and a subpopulation of cells also coexpressed vimentin (Fig. 2i). From day 5 PL, Hsp27 expression became restricted to the glial scar (Fig. 8d), and Hsp27-positive astrocytes maintained vimentin expression (Fig. 2j and k).

Additionally, a small subset of reactive microglial cells located in the margins between the NA and neighboring tissue expressed Hsp27 at day 1 PL (Fig. 2g). Remarkably, no Hsp27-positive microglial cells were observed at any other survival time examined (Fig. 2l).

In the secondarily damaged thalamic nuclei, Hsp27 expression was observed between days 5 and 7 PL. Hsp27 labeling was located in a restricted area of the VB complex (Fig. 8f), close to the neurodegenerative area. Thalamic Hsp27-positive cells were identified as reactive astroglial cells according to their labeling with GFAP. No Hsp27 expression was observed in the CL, although this thalamic nucleus displayed a strong glial reactivity.

DISCUSSION

Constitutive Expression of Hsp27

To our knowledge, the present study describes for the first time Hsp27 constitutive expression in the imma-

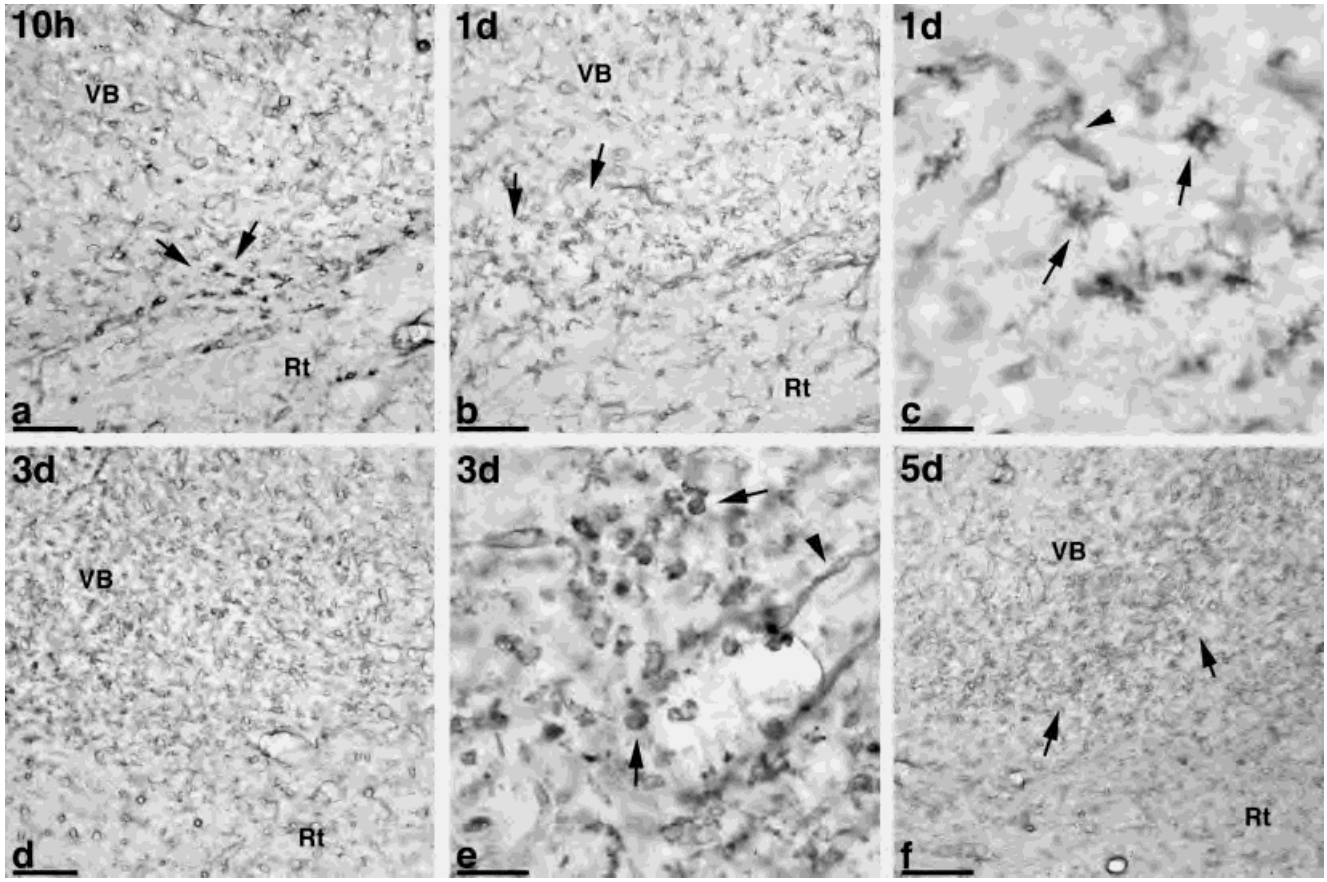

Fig. 6. Micrographs showing the microglial reactivity in the secondarily affected thalamus by means of tomato lectin histochemistry, at 10 h (**a**), day 1 (**b** and **c**), day 3 (**d** and **e**), and day 5 (**f**) PL. Microglial reactivity is first observed at 10 h PL in the inferior third of the VB (arrows in a), increasing thereafter (arrows in b), peaking at day 3 PL (d and e), and decreasing from day 5 PL (arrows in f). Microglial reactivity was characterized by an increase in lectin binding and the presence of amoeboid (arrows in c) and round (arrows in e) microglial morphologies. VB, ventrobasal thalamic complex; Rt, reticular nucleus. Scale bars: a, b, d, and f, 90 μm; c and e, 35 μm.

ture rat brain, mainly in white matter astrocytes located in the internal capsule. Singularly, the developmental evolution of Hsp27 expression in the internal capsule coincided in time with important developmental events in this white matter tract. In this sense, Hsp27 expression paralleled tissue remodeling due to the removal of exuberant axonal connections by reactive microglial cells (Ferrer et al., 1992; Earle and Mitrofanis, 1998), and the decrease in Hsp27 expression observed from P14 correlated with the downregulation of this microglial reactivity (Earle and Mitrofanis, 1998), suggesting an involvement of Hsp27 in the processes triggering developmentally induced tissue remodeling.

Hsp27 Expression After Developmental Traumatic Brain Damage

Following a cortical aspiration lesion in the immature brain, we have described a strong upregulation of Hsp27 expression in a very circumscribed area surrounding the wound. These findings contrast with reports on focal cortical lesions in the adult brain, where increased Hsp27 expression extends throughout the whole ipsilateral cortex (Plumier et al., 1997a, 1997b). Interestingly, these differences on the pattern of Hsp27 expression may be related to the developmental stage. In the rat brain, the second postnatal week of postnatal life has been described as a critical period or a plasticity window (Kolb et al., 1994, 1996) because the brain is particularly neuroprotected due to the presence of high levels of trophic factors (Snyder et al., 1998; Werther et al., 1998) and the constitutive expression of protective molecules such as the antioxidant Hsp32 or heme oxygenase-1 (HO-1) (Bergeron et al., 1998). This particular condition may prevent the extension of the lesion to the distal cortex, resulting in a more restricted area of tissue damage and therefore limiting Hsp27 expression.

At longer survival times, Hsp27 increases in the cavity borders, where the glial scar begins to form. Interestingly, this expression of Hsp27 correlates, in a similar developmental traumatic injury model,

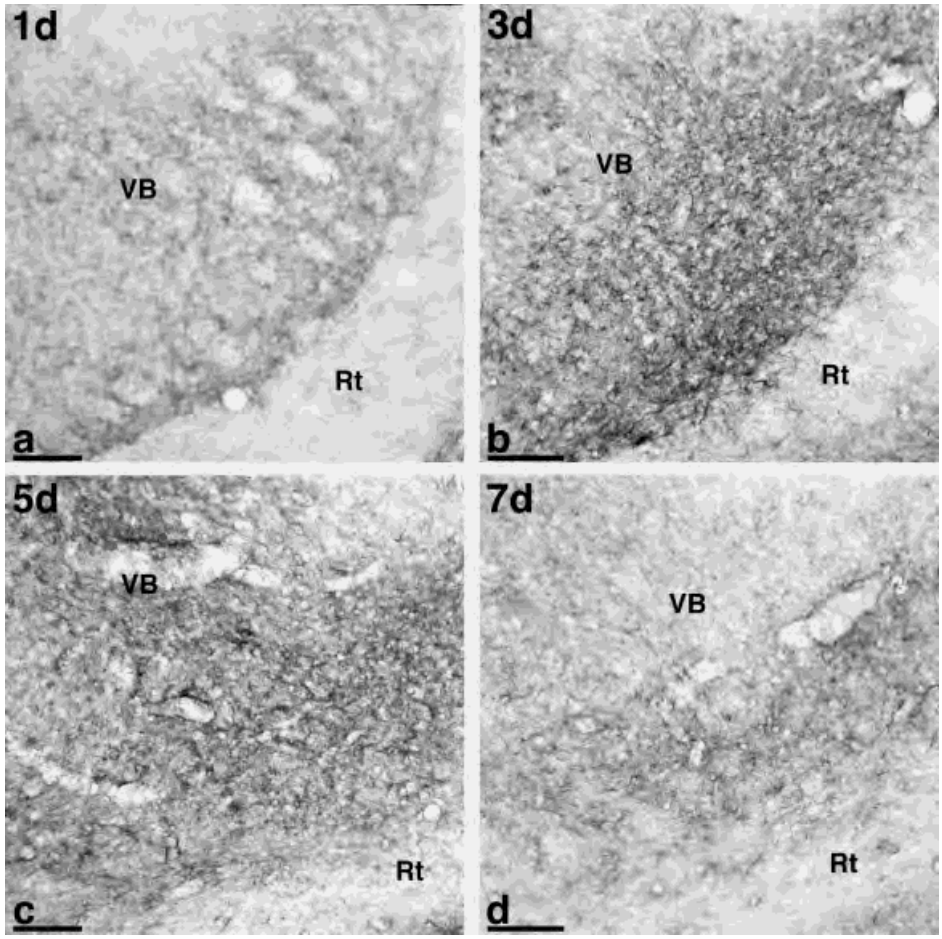

Fig. 7. Micrographs showing the astroglial reactivity in the thalamus by means of GFAP immunohistochemistry, at 1 day PL (**a**), 3 days PL (**b**), 5 days PL (**c**), and 7 days PL (**d**). Thalamic astroglial reactivity was characterized by a strong increase in GFAP immunoreactivity from day 1 PL (a), reaching peak levels between day 3 (b) and day 5 PL (c), and decreasing thereafter (d). VB, ventrobasal thalamic complex; Rt, reticular nucleus. Scale bars, 90 μm.

with an upregulation of basic fibroblast growth factor (bFGF) (Smith and Hale, 1997), which is known to play an important role in glial scar formation (Smith and Hale, 1997). In this sense, Piotrowicz et al. (1997) have reported that Hsp27 is essential for promoting bFGF secretion, suggesting that Hsp27 could play an important role in bFGF-mediated tissue repair and glial scar development.

In addition to the changes in Hsp27 labeling in cortical areas, the secondarily affected thalamus showed a delayed pattern of Hsp27 expression, which was not evident until day 5 PL in the inferior third of the VB complex, where both anterograde terminal degeneration and retrograde neuronal death occur. As no massive tissue loss occurs in the distal thalamus, it is likely that factors released by VB dying neurons may be sufficient for triggering expression of Hsp27 in surrounding astrocytes.

Considering both the primary cortical degenerating area and the secondarily affected thalamus, we have observed Hsp27 induction in areas undergoing anatomical reorganization and axonal sprouting. The expression of Hsp27 in areas surrounding tissue degeneration supports the previously stated hypothesis (Kato et al., 1999) that Hsp27 induction correlates with areas of injury-induced tissue remodeling.

Cellular Localization of Hsp27 and Putative Functions

Following the cortical lesion, Hsp27 expression was upregulated mainly in astrocytes but also in microglial cells to a lesser extent. Accordingly, only a small subpopulation of microglial cells expressed Hsp27 in a pulse-like pattern, being only observed at 1 day PL. Similar findings have been reported after adult brain damage, where different studies have reported a fast and transient microglial Hsp27 expression (Plumier et al., 1997a, 1997b; Brown and Sharp, 1999). Although little is known about the role of Hsp27 in the microglial response, its characteristic pulse-like induction may suggest an involvement of Hsp27 in a specific stage of microglial cell changes to stress, probably including cytoskeletal changes.

Besides this transient microglial Hsp27 induction, reactive astrocytes are the main cell type expressing Hsp27. In astroglial cells, Hsp27 colocalizes with the cytoskeletal proteins GFAP and vimentin in the major thick astroglial processes. In this regard, a recent in vitro study has suggested an interaction of Hsp27 with intermediate filaments, protecting them from stress-induced aggregation (Perng et al., 1999). Therefore, the in vivo colocalization of Hsp27 with GFAP and vimen-

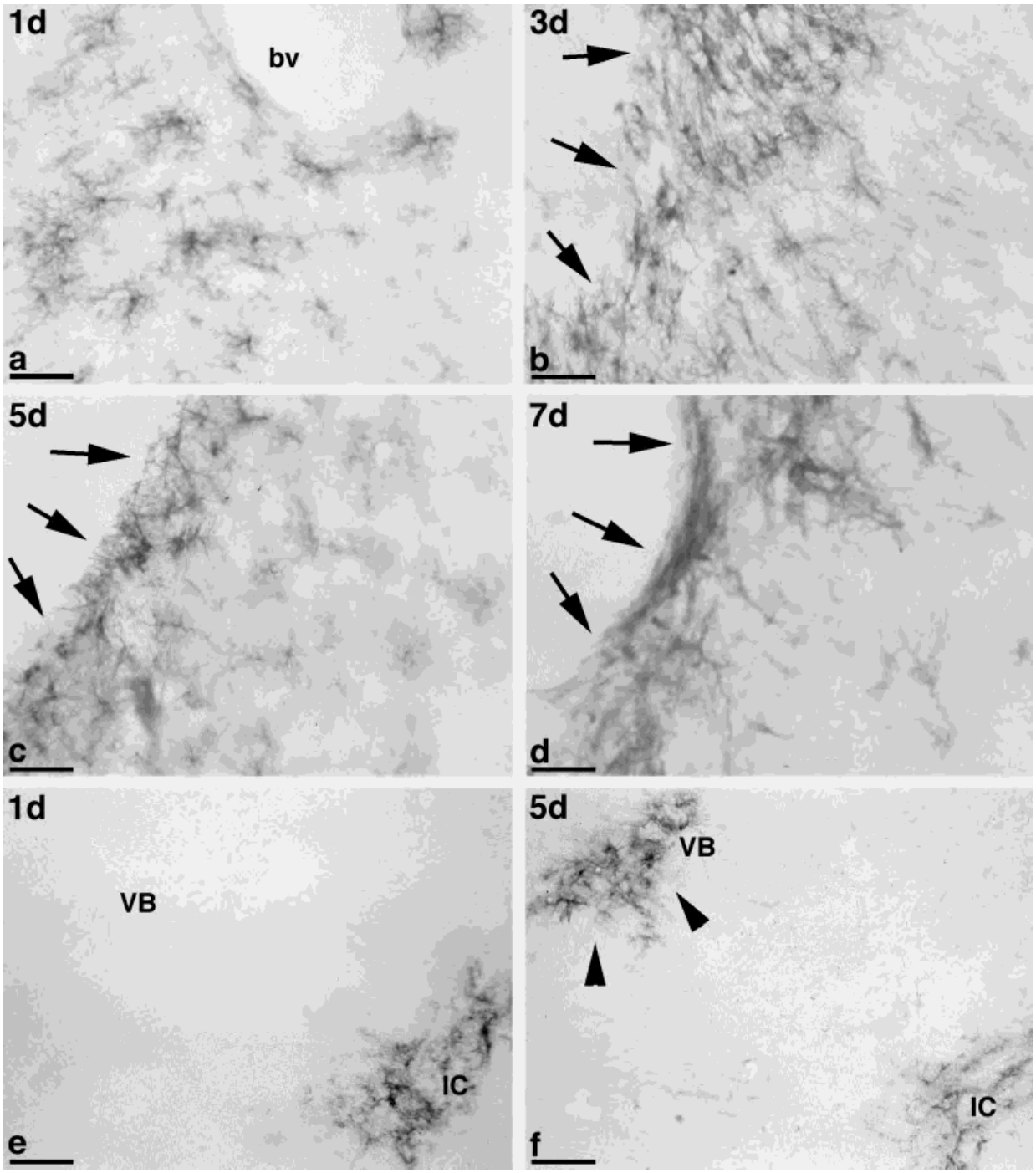

Fig. 8. Micrographs showing Hsp27 expression in the cortical (**a**–**d**) and thalamic (**e** and **f**) injured areas, at day 1 (a and e), day 3 (b), day 5 (c and f), and day 7 (d) PL. In the cortex, Hsp27 is first observed at 1 day PL (a; bv, blood vessel) in highly ramified astrocytes (high magnification square in a). Hsp27 immunoreactivity is further increased at longer survival times (b–d), accumulating in the glial scar at day 7 PL (d). Arrows in b–d show the borders of the cavity. In the thalamus, Hsp27 is only observed in the internal capsule (IC) at early time points (e) and in the degenerating ventrobasal complex (VB) from 5 days PL (arrowheads in f). Scale bars: a–c, 35 μm; d, 25 μm; e and f, 90 μm.

tin could be interpreted as a protective mechanism to maintain astroglial cytoskeleton integrity, thus promoting astroglial survival. Furthermore, several evidences suggest that Hsp27 shares some functions with vimentin; both proteins have been involved in intracellular transport (Pixley and De Vellis, 1984; Piotrowicz et al., 1997) and cellular motility (Schiffer et al., 1986; Piotrowicz et al., 1998), and both have been proposed to provide a link between membrane signaling mechanisms and cytoskeleton (Hutchins and Casagrande,

1989; Lavoie et al., 1993). These findings may indicate that the interaction between Hsp27 and vimentin could imply a cooperation of these proteins in the previously mentioned functions, in addition to the cytoskeletal protective role of Hsp27 discussed above.

Moreover, another postulated function of Hsp27 is to provide antioxidant mechanisms. In vitro studies have suggested a protective function of Hsp27 against TNF-α–induced oxidative stress through an increase in glutathione levels (Mehlen et al., 1996a, 1997). Furthermore, a recent study has pointed out that Hsp27 accumulation correlates with the restoration of glutathione levels following kainic acid injection (Kato et al., 1999), suggesting that, after brain damage, Hsp27 would act as an additional astroglial antioxidant in the same way as those already described for astrocytes (Makar et al., 1994; Peuchen et al., 1997). Finally, this wide range of functions attributed to Hsp27 suggests that this protein may not only protect the cells where it is expressed, but also influence the surrounding tissue by decreasing its damage and inducing its restoration.

In conclusion, we have demonstrated that astrocytes are the main cell type expressing Hsp27 in the postnatal brain, both in basal conditions and after injury. Hsp27 is induced in reactive astroglial cells located in areas of cortical traumatic injury as well as in the distally connected thalamus. These results point to Hsp27 as a good marker for areas undergoing tissue remodeling after damage and may suggest a possible protective role of this cytoskeleton-stabilizing heat shock protein in tissue reorganization in the immature brain.

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