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Quantitative Analysis of Microglial Reaction to a Cortical Excitotoxic Lesion in the Early Postnatal Brain

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This study was designed to quantify the microglial response following an injection of *N*-methyl-D-aspartate (NMDA) into the sensorimotor cortex of 6-day-old rats. After survival times ranging from 10 h to 28 days, cryostat sections were processed for the demonstration of microglial cells by means of tomato lectin histochemistry. The injection of NMDA caused an extensive primary lesion involving the neocortex, the rostral hippocampus, and rostral thalamus. In addition, secondary retrograde/anterograde degeneration was also observed in the ventrobasal (VB) complex of the thalamus. Microglial reactivity was already present at 10 h postlesion and restricted to areas of neuronal degeneration. Quantitative analysis was performed on digitized images using NIH Image software and a Macintosh computer. The method is based on densitometric ratios, referred to as the "reactivity grade," between the ipsilateral lesion side and the contralateral control side. Measurements were made to determine a possible increase in the number of microglial cells as well as an increase in lectin binding. The analysis showed that microglial reactivity in areas of primary degeneration peaked at 3 days postlesion, when it was significantly ($P < 0.01$) higher in comparison to saline-injected litter mates. Microglial response in the cerebral neocortex, showing the highest reactivity grade, as well as in other areas of primary degeneration, returned to control levels by Day 7. Microglial response in the VB complex also peaked at Day 3 ($P < 0.05$) but maintained this level of reactivity until 7 days postlesion ($P < 0.01$). © 1997 Academic Press

INTRODUCTION

Lesions in the nervous system can cause axonal and/or neuronal degeneration and an accompanying glial response in the primary lesion area as well as in secondary distal areas neuroanatomically connected with the lesion site (17, 40). Several models have been used to examine astroglial and microglial changes in secondary degenerating areas; these include the study of retrograde changes in the facial motor nucleus after damage of the facial nerve by axotomy or by ricin injection (14, 15, 36, 37) or the analysis of axonal anterograde changes in the hippocampal fascia dentata following entorhinal cortical lesions (8, 11, 19). The thalamic atrophy that occurs following cortical injury has also been examined (17, 21, 26, 32–34). These studies primarily have focused on the morphological description or provide a functional analysis of the glial response, usually a temporal delay of the glial response in distal areas is observed. In order to expand information given by qualitative observations, quantitative data giving a more objective and accurate measure of the different pattern and timecourse of glial response in primary degenerating areas versus secondary degenerating areas is needed. Although some attempts to quantify glial response in other lesion models have already been made (5, 16, 30), these reports do not provide information refering to all of the different aspects of the gliosis, such as increase in marker binding, cell hypertrophy, and hyperplasia.

In a recent work we have presented a morphological analysis of the microglial reaction to an excitotoxic lesion in the sensorimotor cortex of immature rats, focusing on primary degenerating areas as well as the secondary response within the ventrobasal (VB) complex of the thalamus, where anterograde and retrograde degeneration occurs (1). An important issue toward understanding the role and implication of the microglial response after excitotoxic and other types of brain injury concerns the changes in the intensity of this glial response. Therefore, the aim of the present study was to provide an important quantitative complement to morphological observations using an easy and reliable method to quantify all aspects of the microglial response.

MATERIALS AND METHODS

Excitotoxic Lesions

Long Evans black-hooded 6-day-old rats of both sexes (day of birth equals Day 0) received a unilateral injection of NMDA (50 nmol diluted in 0.2 μl of saline

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solution) into the right somatosensory cortex, as described previously (1). After surgery, the pups were placed in an incubator and maintained at 36°C for 2 h. Control animals followed the same procedure, but received an injection of 0.2 µl of saline solution. Four NMDA-injected animals and two saline-injected control animals were used for each of six postinjection survival times.

Fixation and Lectin Histochemistry

At 10 h, 1 day, 3 days, 7 days, 14 days, and 28 days after NMDA or saline injection, rats were perfused intracardially and postfixed with 4% paraformaldehyde in 0.1 *M* phosphate buffer (pH 7.4). Brains were immediately removed, quickly frozen with CO_2, and stored at −70°C until sectioned in a cryostat. Floating frozen sections (30–40 µm thick) were processed for tomato lectin histochemistry for the visualization of microglial cells, as described previously (2). Briefly, endogenous peroxidase was blocked with 2% H_2O_2 in 100% methanol, sections were rinsed, and incubated for 2 h at room temperature with the biotinylated tomato lectin (*Lycopersicon esculentum*) (Sigma L-9389) diluted to 6 µg/ml. After incubation, sections were washed in buffer and incubated for 1 h at room temperature with avidin peroxidase (Sigma, A-3151) in a 1:70 dilution. The peroxidase reaction product was visualized using 3′-diaminobezidine (DAB). Finally, the sections were rinsed, mounted on gelatin-coated slides, dehydrated, cleared in xylene, and coverslipped in DPX. Control of tomato lectin specificity was carried out by incubating tomato lectin for 30 min in a 0.1 *M* solution of *N*-acetyllactosamine (Sigma, A-7791), sugar to which the lectin binds, and incubating the sections in the lectin solution with the same sugar.

Quantification Method

Histological sections were digitized by a videocamera mounted on a Leitz microscope and interfaced to a Macintosh computer. National Institute of Health Image software (NIH 1.52) was used to quantify the histochemical labeling of microglial cells. The quantification measure, referred to as the "reactivity grade," was defined as the ratio between values of a specific area in the cerebral hemisphere ipsilateral to the NMDA injection versus its homologous area in the contralateral control hemisphere. In order to obtain this ratio, we have developed an equation consisting of three factors:

(i) The first factor is the quotient between the ipsi- and contralateral densities of DAB reaction product (mean staining density). This factor shows the increase in lectin binding in the ipsilateral hemisphere and compensates for the normal variability in the intensity of DAB reaction product between sections. Moreover, the mean density of the contralateral control hemisphere is chosen as the threshold value and allows us to calculate the next factor.

(ii) The second factor is the quotient of the ipsi- and contralateral areas above the threshold. This parameter considers the possible increase of the area above threshold in the ipsilateral hemisphere due to microglial hypertrophy and/or hyperplasia.

(iii) The third factor is a correction term to compensate for the possible shrinkage of degenerating structures in relation to the contralateral control hemisphere.

The specific equation used to calculate the reactivity grade is summarized as follows:

$$\text{Reactivity grade} = \frac{\text{Mean staining density}_{\text{Ipsilat.}}}{\text{Mean staining density}_{\text{Contralat.}}\ \text{(Threshold)}} \times \frac{\text{Area above threshold}_{\text{Ipsilat.}}}{\text{Area above threshold}_{\text{Contralat.}}} \times \frac{\text{Total Area}_{\text{Ipsilat.}}}{\text{Total area}_{\text{Contralat.}}}$$

Factor considering a possible increase in microglial lectin binding

Factor considering possible microglial hyperplasia and/or hypertrophia

Correction factor to compensate for a possible shrinkage of degenerating areas

For easier understanding, a graphic example of this quantification method is presented in Fig. 1.

A reactivity grade was obtained for each area of study (i.e., injured area of the neocortex, damaged rostral hippocampus and rostral thalamus, and secondarily affected VB complex of the thalamus) and for each animal (mean of two sections). Four animals for each NMDA-injected group were analyzed. The same measurements were done in saline-injected animals. Measured areas as shown in Fig. 2 included: (i) an area of 1 mm^2 in the neocortex at the level of the injection site; (ii) the entire cross-sectional area of the hippocampus; (iii) the rostral thalamus, including all nuclei; and (iv) 2.63-mm^2 area of the caudal thalamus, including the VB complex, reticular nuclei, and the internal capsule. Thalamic nuclei were identified according to Robertson and coworkers (31). Statistical analysis was performed using Statview 4.0 software. Analysis of variance (ANOVA) with Fisher's PLSD post-hoc comparisons were used.

RESULTS

As described previously (1), NMDA injection into the right sensorimotor cortex of 6-day-old rats caused an extensive primary lesion usually involving the neocortex, the dorsal striatum, the dorsal hippocampus, and the rostral thalamus. These areas were neuron depleted by 1 day after the lesion. Neuronal swelling and degeneration was also observed in the VB thalamic

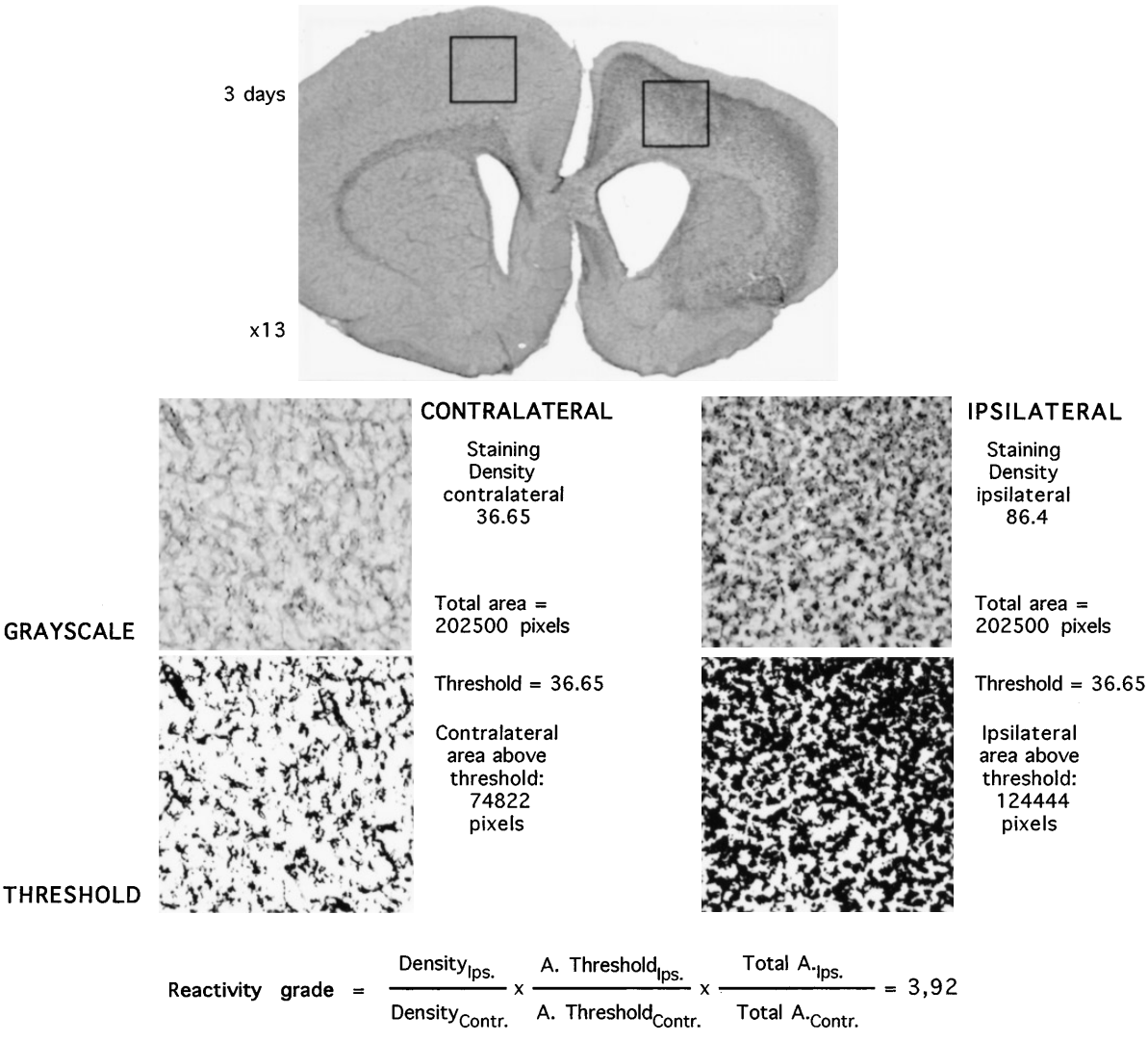

FIG. 1. Graphic representation of the quantification method as seen in the NIH Image system. Final reactivity grade is obtained following the formula, as explained under Materials and Methods.

complex. No apparent degeneration was observed in the contralateral hemisphere.

In those animals receiving NMDA injections, tomato lectin histochemistry allowed the visualization of lesion-induced activated microglial cells (referred to as reactive microglial cells). Ameboid and ramified microglia observed in control brains are also visualized using tomato lectin histochemistry. Blood vessels and ependymal cells were also stained. Microglial reactivity was restricted to degenerating areas, present in the neocortex (at the level of the injection site and at more caudal levels) (Figs. 3A and 3B) and the rostral hippocampus as well as the lateroposterior (LP), laterodorsal (LD), and reticular-ventroanterior transition (R-VA) nuclei of the rostral thalamus (Figs. 3C and 3D). In addition, reactive microglial cells were also observed in the VB thalamus (Figs. 3E and 3F), this microglial response could be attributed to secondary neuronal degeneration caused by cortical target depletion. Microglial reactivity was restricted to the areas undergoing neuronal or axonal degeneration. Morphological changes of microglial cells were already observed at 10 h postinjection. Maximal microglial reactivity was apparent 3 days postinjection, when reactive microglial cells showed intense lectin staining and round or pseudopodic morphology. By 7 days the lesion became nonprogressive, and at 14 and 28 days postinjection microglial cells showed moderate lectin binding and a more ramified morphology.

Quantification Analysis

Quantification of microglial reactivity is presented in Table 1 and graphically represented in Fig. 4. Global results of all areas in a real time scale are shown in Fig. 5. Saline-injected animals showed an overall mean

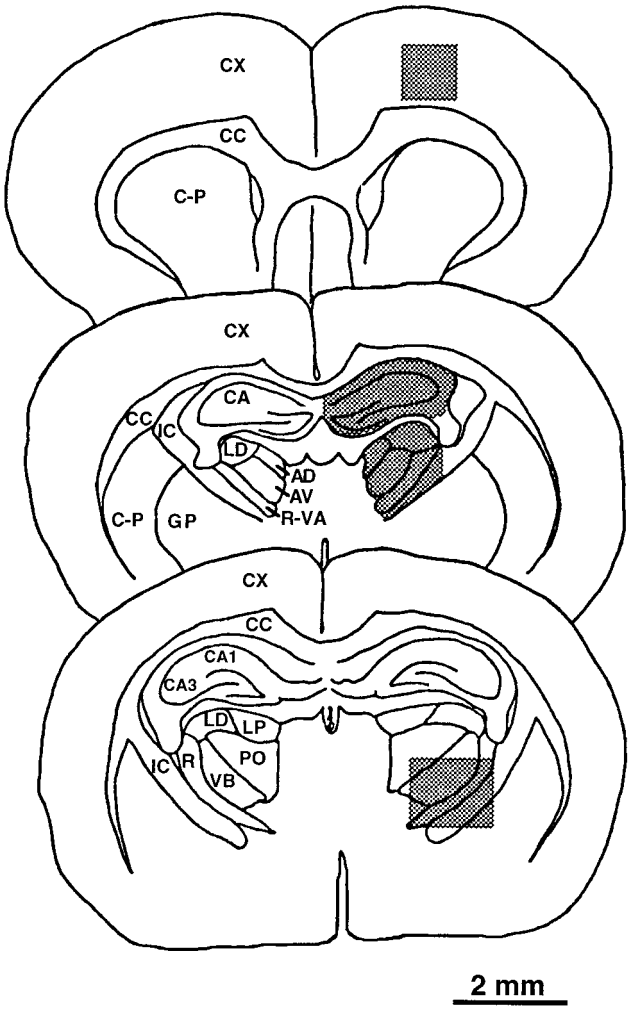

FIG. 2. Camera lucida drawing showing measured areas (shadowed) in the cortex, the hippocampus, the rostral thalamus, and the caudal thalamus. Abbreviations: AD, anterodorsal thalamic nuclei; AV, anteroventral thalamic nucleus; CA, cornus ammonis; CC, corpus callosum; C-P, caudate-putamen; CX, cerebral cortex; GP, globus pallidus, IC, internal capsule; LD, laterodorsal thalamic nucleus; LP, lateroposterior thalamic nucleus; PO, posterior thalamic nucleus; R, reticular nuclei; R-VA, ventroanterior-reticular nuclei transition; VB, ventrobasal complex of the thalamus.

reactivity grade of 1.039 ± 0.007 (mean of all areas and all times $\pm$ SD), indicating equality between the ipsilateral and the contralateral sides of the brain. In NMDA-injected animals, a slight increase in microglial reactivity was already seen at 1 day postinjection, especially in the cortical injection site, with a reactivity grade value of 1.71. The increase in microglial reactivity was significantly higher than saline-injected littermates at 3 days postinjection with peak values of 3.5 in the cortex (injection site) ($P < 0.01$), 2.32 in the rostral thalamus, and 1.85 in the VB complex ($P < 0.05$ in these last areas). Microglial reactivity grades in the hippocampus did not achieve significance at any survival time. The highest reactivity grade was found at Day 3 in the cortical injection site, indicating a microglial response that was 3.5-fold higher than the contralateral cortex.

The microglial reactivity grade in the neocortex and rostral thalamus decreased to nonsignificant values from Day 7 postinjection. However, the maximal reactivity grade found in the VB complex persisted at a level that was statistically higher ($P < 0.01$) than the same area in saline-injected littermates until 7 days after NMDA injection. This prolonged thalamic microglial reactivity most likely corresponds to the secondary thalamic degeneration that occurs after cortical lesions.

DISCUSSION

The aim of the present work was to quantify by densitometric methods the microglial reaction found in areas of primary and secondary (retrograde/anterograde) neuronal degeneration following an excitotoxic cortical lesion in the early postnatal rat brain. This reactivity was generally more prominent in the primary lesion areas, directly affected by the injection, than in the thalamic VB nuclei undergoing distal secondary degeneration. However, the secondary degenerating areas showed a persistent peak of microglial response until 7 days after the lesion. This protracted microglial response was not found elsewhere.

While these quantification findings correlate in general with morphological observations, the first signs of microglial response, reflected by morphological changes toward ameboid and pseudopodic forms, were already obvious at 10 h and 1 day after the injection, even though a statistically significant increase in the reactivity grade was not found. In this sense, normal blood vessel lectin staining may obscure qualitatively observed microglial activation, but this measure only makes the procedure more stringent.

Our quantification appeared to be very useful to evaluate microglial reactivity intensity and time-course, and to compare different brain areas, especially in the early postnatal brain, where postlesion glial reactivity occurs very fast and decreases in a very short period of time (26). Furthermore, the quantification method used revealed a protracted thalamic microglial response which was not so apparent upon routine histological analysis.

Quantification of a glial response has generally been very tedious because of the difficulty in identifying single reactive cells in areas of massive gliosis and the problem to express numerically an increase in glial markers labeling. In view of this difficulty, most previous studies have used semiquantitative scales (3, 7, 9, 11, 16, 20, 28, 29, 35) in order to express the different levels of the glial response. Although based on subjective measures, such fairly easy methods can add to the interpretation of histological data. In an effort to quan-

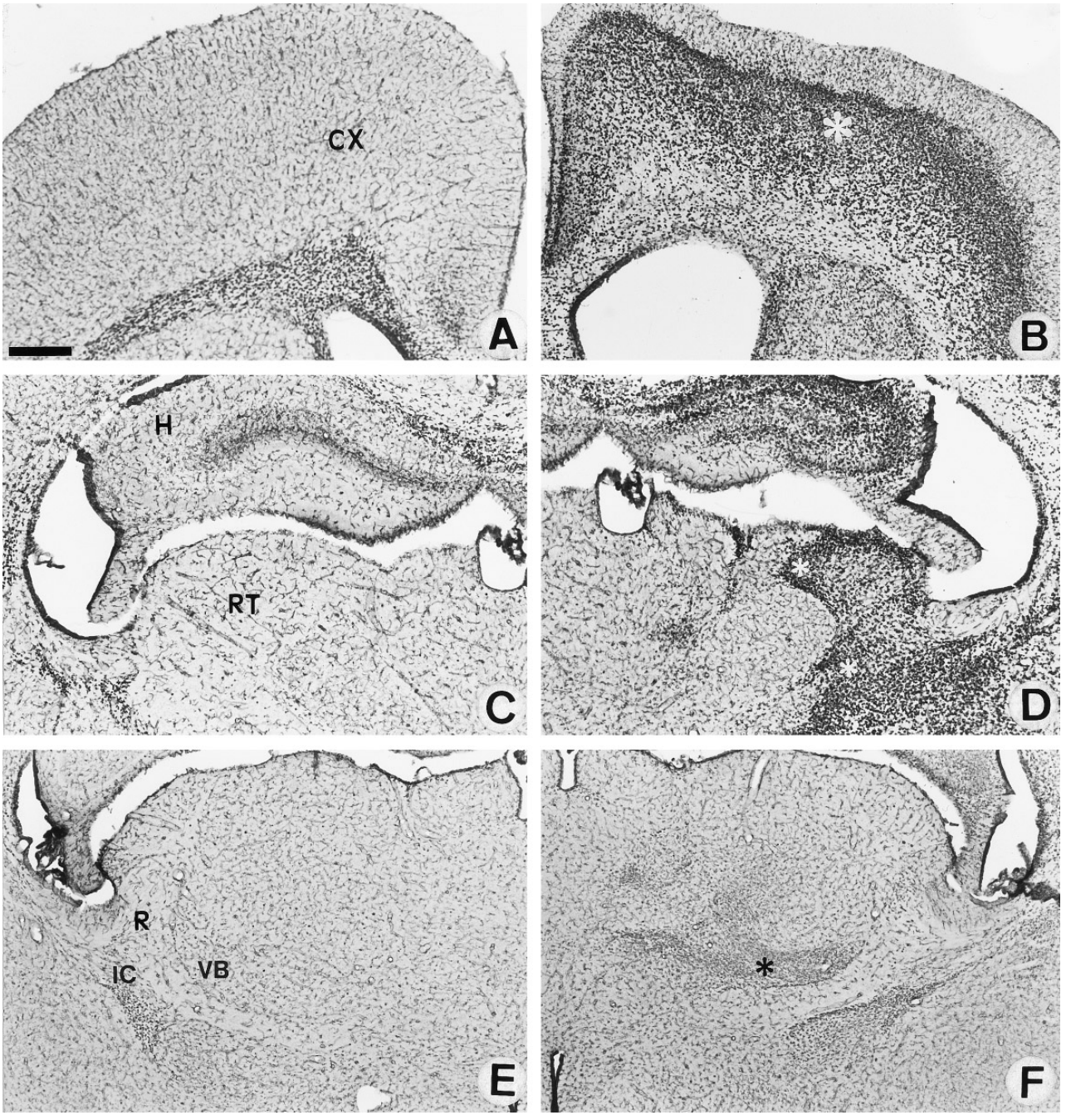

FIG. 3. Micrographs of contralateral (A, C, E) and ipsilateral (B, D, F) hemispheres of tomato lectin-stained sections 3 days after right neocortical NMDA injection. Cortex at the level of the injection site (A, B); hippocampus and rostral thalamus (C, D); and VB complex (E, F). Note the sharp border between areas of microglial reactivity (marked with asterisks) and nonreactive areas. Abbreviations: CX, cortex; IC, internal capsule; R, reticular nuclei; RT, rostral thalamus; VB, ventrobasal complex of the thalamus. Scale bar, 500 μm.

tify the astroglial response, some reports have provided areal measures of glial fibrillary acidic protein (GFAP) immunoreactivity (3, 24).

Although cell counts provide the best method to evaluate an increase in reactive glial cell number, such methods provide no information on the increase in marker binding or cell hypertrophy, common characteristics of reactive glial cells. The quantification analysis described in this paper is useful in the sense that it provides a numeric value of the microglial reactivity taking into account all aspects of the response of glial cells to injury (increased marker binding, hypertrophy, and hyperplasia), thus reflecting more acutely the observations under the microscope. Counting of whole

TABLE 1

Reactivity Grades

	Cortex	Hippocampus	Rostral thalamus	VB Complex thalamus
Saline control[a]	1.100 ± 0.05	1.102 ± 0.220	1.004 ± 0.264	0.948 ± 0.071
10 h	1.300 ± 0.285	1.235 ± 0.322	1.141 ± 0.150	1.147 ± 0.116
1 day	1.710 ± 0.275	1.032 ± 0.131	1.212 ± 0.096	1.261 ± 0.073
3 days	3.502** ± 0.418	1.044 ± 0.139	2.325* ± 0.475	1.852* ± 0.193
7 days	1.689 ± 0.223	0.682 ± 0.151	0.916 ± 0.126	1.853** ± 0.257
14 days	1.263 ± 0.120	0.870 ± 0.098	1.032 ± 0.095	1.071 ± 0.031
28 days	1.437 ± 0.071	1.069 ± 0.276	0.930 ± 0.019	1.062 ± 0.071

Note. Microglial reactivity grades of all areas at different survival times. Values are means ± SEM. * $P < 0.05$; ** $P < 0.01$ as compared to values in saline controls (ANOVA and Fisher PLSD test).

[a] Saline values are mean grades of all survival times for each area ± SD.

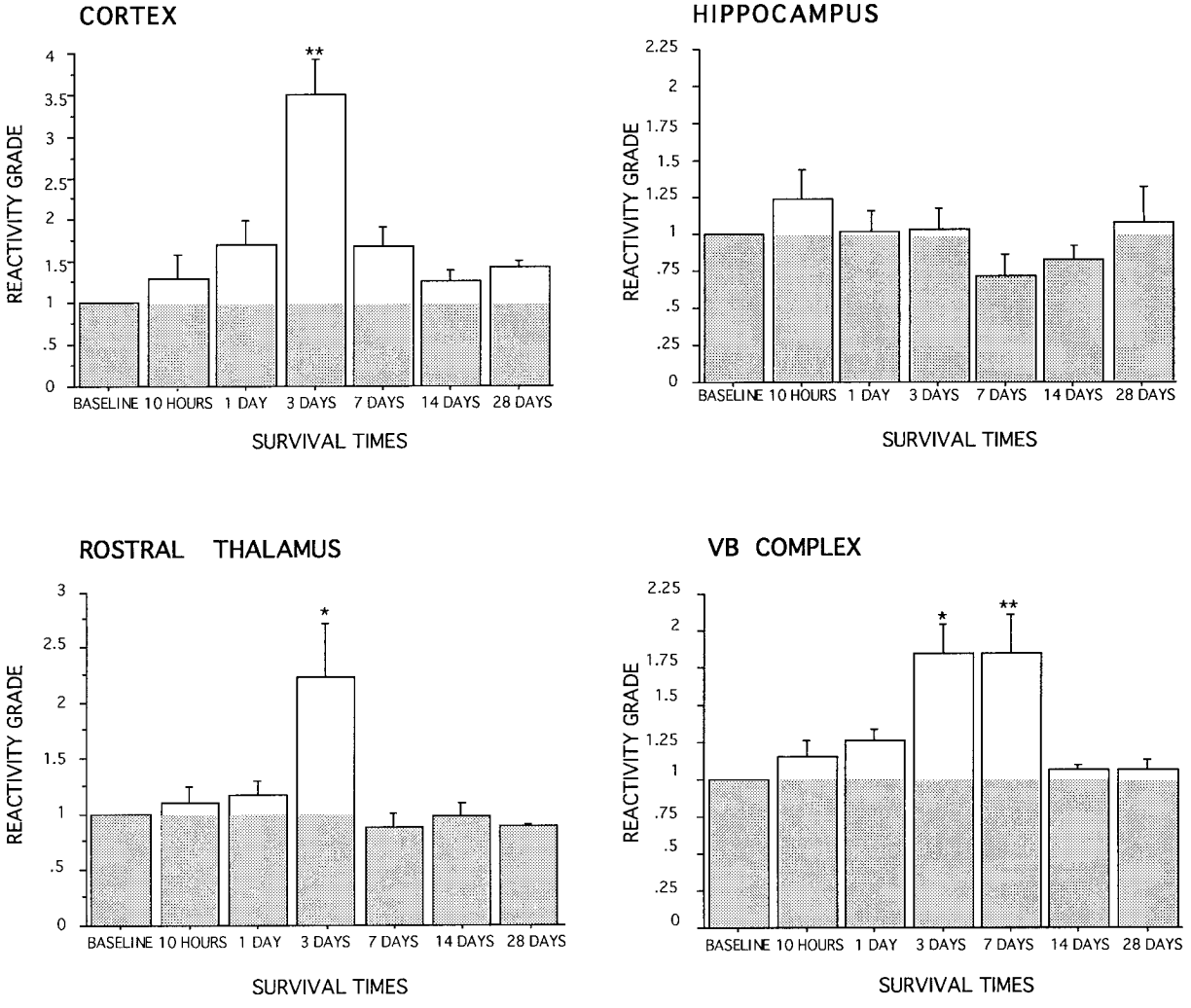

FIG. 4. Graphic representation of microglial reactivity quantification, in the areas studied at several survival times after the NMDA injection. Data are presented as the mean values of reactivity grades ± SEM. Significative values are obtained comparing with saline-injected littermates of each survival time (**$P < 0.01$; *$P < 0.05$). Equivalence between hemispheres (reactivity grade = 1) is defined as baseline and is shadowed for better understanding. Significant increase of the reactivity grade was observed in the neocortex, rostral thalamus, and VB complex 3 days after the injection. Significance only persisted till Day 7 in the VB complex.

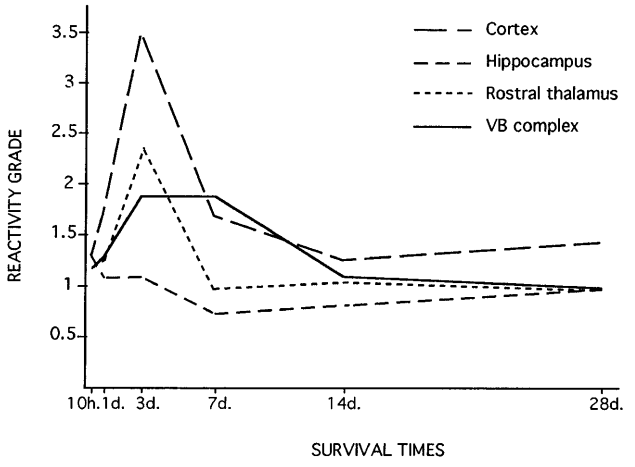

FIG. 5. Summary graph showing microglial reactivity grades in all the areas analyzed and all survival points in a time scale. Note that reactivity in the VB complex is weaker but remains high till 7 days postlesion.

astroglial or microglial cell populations, in addition to being especially tedious and therefore scarce, can only be done where single cells are easily delimited, such as the counting of amoeboid microglial cells during normal development (41, 42), of resting microglial cells in the mouse cerebellum (39), or the microglial population in the jimpy mutant mouse spinal cord (38). To our knowledge there are no studies counting reactive microglial cells after brain lesions, where the high density of reactive cells and the proximity between them makes it impossible to reliably distinguish single cell bodies. Alternative methods that have counted the total number of nonneuronal cells nuclei in sections stained for routine histology (4, 23) cannot provide distinction between astroglial, microglial, and oligodendroglial cells.

Several studies have reported cell counts of glial subpopulations identified by specific markers such as major histocompatibility complexes I or II antibodies (22, 43), leukocyte common antigen antibody (22, 25), macrophage markers (like ED-1) (22), growth factors markers (13), or proliferation markers like [^{3}H]thymidine, 5′-bromodeoxyuridine or proliferating cell nuclear antigen (PCNA) antibody (6, 10, 12, 18, 27). Cell counts determined by these studies have provided useful and important information, but these methods are unable to quantify the whole glial response.

Ingenious methods using image analysis have been developed in an effort to quantify overall glial reactivity. For example, semiquantitative analysis has involved measures of optical density of GFAP immunoreactivity (30), and more recent work used similar methods to analyze microglial reactivity in areas showing OX42 immunoreactivity (5). In comparison, the quantification method used in the present study provides an objective measure of microglial cell number increase, enlargement of microglial cell bodies, and increase in lectin binding. The reactivity grade value as such is a representative measure of microglial reactivity, without distinguishing the contribution provided by each parameter, being more accurate in describing the timecourse of the microglial response in the differently affected areas than independent values of higher lectin binding or increased cell number. Quantification of the glial response may be an important point to evaluate possible changes after different kinds of injury like excitotoxicity and following putative protective treatments such as the application of NMDA receptor antagonists or free radical scavengers. The analysis of quantitative differences in the levels of these glial reactions may give some insights into the role or implication of these cells after brain damage.

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REFERENCES

1. Acarin, L., B. Gonzalez, B. Castellano, and A. J. Castro. 1996. Microglial response to *N*-methyl-D-aspartate (NMDA) mediated excitotoxicity in the immature rat brain. *J. Comp. Neurol.* **367:** 361–374.
2. Acarin, L., J. M. Vela, B. Gonzalez, and B. Castellano. 1994. Demonstration of poly-*N*-acetyl lactosamine residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. *J. Histochem. Cytochem.* **42:** 1033–1041.
3. Balasingam, V., T. Tejadaberges, E. Wright, R. Bouckova, and V. W. Yong. 1994. Reactive astrogliosis in the neonatal mouse brain and its modulation by cytokines. *J. Neurosci.* **14:** 846–856.
4. Barron, K. D., F. F. Marciano, R. Amundson, and R. Mankes. 1990. Perineuronal glial responses after axotomy of the central and peripheral axons. A comparison. *Brain Res.* **523:** 219–229.
5. Eriksson, N. P., J. K. Persson, M. Svensson, J. Arvidsson, C. Molander, and H. Aldskogius. 1993. A quantitative analysis of the microglial cell reaction in central primary sensory projection territories following peripheral nerve injury in the adult rat. *Exp. Brain Res.* **96:** 19–27.
6. Fagan, A. M., and F. H. Gage. 1994. Mechanisms of sprouting in the adult central nervous system—cellular responses in areas of terminal degeneration and reinnervation in the rat hippocampus. *Neuroscience* **58:** 705–725.
7. Fernaud-Espinosa, I., M. Nieto Sampedro, and P. Bovolenta. 1993. Differential activation of microglia and astrocytes in aniso- and isomorphic gliotic tissue. *Glia* **8:** 277–291.
8. Finsen, B. R., N. Tonder, G. F. Xavier, J. C. Sorensen, and J. Zimmer. 1993. Induction of microglial immunomolecules by anterogradely degenerating mossy fibres in the rat hippocampal formation. *J. Chem. Neuroanat.* **6:** 267–275.
9. Flaris, N. A., T. L. Densmore, M. C. Molleston, and W. F. Hickey. 1993. Characterization of microglia and macrophages in the central nervous system of rats: Definition of the differential

expression of molecules using standard and novel monoclonal antibodies in normal CNS and in four models of parenchymal reaction. *Glia* **7:** 34–40.

10. Garcia Estrada, J., J. A. Del Rio, S. Luquin, E. Soriano, and L. M. Garcia Segura. 1993. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. *Brain Res.* **628:** 271–278.
11. Gehrmann, J., S. W. Schoen, and G. W. Kreutzberg. 1991. Lesion of the rat entorhinal cortex leads to a rapid microglial reaction in the dentate gyrus. A light and electron microscopical study. *Acta Neuropathol.* **82:** 442–455.
12. Giordana, M. T., A. Attanasio, P. Cavalla, A. Migheli, M. C. Vigliani, and D. Schiffer. 1994. Reactive Cell Proliferation and Microglia Following Injury to the Rat Brain. *Neuropathol. Appl. Neurobiol.* **20:** 163–174.
13. Gomez-Pinilla, F., J. W.-K. Lee, and C. Cotman. 1992. Basic FGF in adult brain: cellular distribution and response to entorhinal lesion and fimbria-fornix transection. *J. Neurosci.* **12:** 345–355.
14. Graeber, M. B., and G. W. Kreutzberg. 1986. Astrocytes increase in glial fibrillary acidic protein during retrograde changes of facial motor neurons. *J. Neurocytol.* **15:** 363–373.
15. Graeber, M. B., W. J. Streit, and G. W. Kreutzberg. 1988. Axotomy of the rat facial nerve leads to increased CR3 complement receptor expression by activated microglial cells. *J. Neurosci. Res.* **21:** 18–24.
16. Herrera, D. G., and A. C. Cuello. 1992. Glial fibrillary acidic protein immunoreactivity following cortical devascularizing lesion. *Neuroscience* **49:** 781–791.
17. Iizuka, H., K. Sakatani, and W. Young. 1990. Neural damage in the rat thalamus after cortical infarcts. *Stroke* **21:** 790–794.
18. Janeczko, K. 1988. The proliferative response of astrocytes to injury in the neonatal brain. A combined immunocytochemical and autoradiographic study. *Brain Res.* **456:** 280–285.
19. Jensen, M. B., B. Gonzalez, B. Castellano, and J. Zimmer. 1994. Microglial and astroglial reactions to anterograde axonal degeneration: A histochemical and immunocytochemical study of the adult rat fascia dentata after entorhinal perforant path lesions. *Exp. Brain Res.* **98:** 245–260.
20. Jorgensen, M. B., B. R. Finsen, M. B. Jensen, B. Castellano, N. H. Diemer, and J. Zimmer. 1993. Microglial and astroglial reactions to ischemic and kainic acid-induced lesions of the adult rat hippocampus. *Exp. Neurol.* **120:** 70–88.
21. Kaur, C., and E. A. Ling. 1992. Activation and re-expression of surface antigen in microglia following an epidural application of kainic acid in the rat brain. *J. Anat.* **180:** 333–342.
22. Lassmann, H., M. Schmied, K. Vass, and W. F. Hickey. 1993. Bone marrow derived elements and resident microglia in brain inflammation. *Glia* **7:** 19–24.
23. Marty, S., I. Dusart, and M. Peschanski. 1991. Glial changes following an excitotoxic lesion in the CNS-I. Microglia/macrophages. *Neuroscience* **45:** 529–539.
24. Mathewson, A. J., and M. Berry. 1985. Observations on the astrocyte response to a cerebral stab wound in adult rats. *Brain Res.* **327:** 61–69.
25. McGeer, P. L., T. Kawamata, D. G. Walker, H. Akiyama, I. Tooyama, and E. G. McGeer. 1993. Microglia in degenerative neurological disease. *Glia* **7:** 84–92.
26. Milligan, C. E., P. Levitt, and T. J. Cunningham. 1991. Brain macrophages and microglia respond differently to lesions of the developing and adult visual system. *J. Comp. Neurol.* **314:** 136–146.
27. Miyake, T., M. Okada, and T. Kitamura. 1992. Reactive proliferation of astrocytes studied by immunohistochemistry for proliferating cell nuclear antigen. *Brain Res.* **590:** 300–302.
28. Molleston, M. C., M. L. Thomas, and W. F. Hickey. 1993. Novel major histocompatibility complex expression by microglia and site-specific experimental allergic encephalomyelitis lesions in the rat central nervous system after optic nerve transection. *Adv. Neurol.* **59:** 337–348.
29. Petito, C. K., M. Chung, I. A. Halaby, and A. J. L. Cooper. 1992. Influence of the neuronal environment on the pattern of reactive astrocytosis following cerebral ischemia. *Prog. Brain Res.* **94:** 381–387.
30. Petito, C. K., S. Morgello, J. C. Felix, and M. L. Lesser. 1990. The two patterns of reactive astrocytosis in postischemic rat brain. *J. Cereb. Blood Flow Metab.* **10:** 850–859.
31. Robertson, R. T., and F. Mostamand. 1988. Development of 'non-specific' choliesterase-containing neurons in the dorsal thalamus of the rat. *Dev. Brain Res.* **41:** 43–60.
32. Ross, D. T., and F. F. Ebner. 1990. Thalamic retrograde degeneration following cortical injury: an excitotoxic process? *Neuroscience* **35:** 525–550.
33. Schulz, M. K., L. Acarin, G. L. Tillotson, and A. J. Castro. 1993. Glial response to neocortical injection of the excitotoxin N-methyl-D-aspartic acid (NMDA). *Soc. Neurosci. Abstr.* **19:** 671.
34. Sorensen, J. C., B. R. Finsen, I. Dalmau, and J. Zimmer. 1992. Microglial reactions and tracer identified thalamic nuclei after frontal cortex lesions in adult rats. *Eur. J. Neurosci. (Suppl.)* **5:** 29.
35. Strauss, S., U. Otten, B. Joggerst, K. Pluss, and B. Volk. 1994. Increased Levels of Nerve Growth Factor (NGF) Protein and Mrna and Reactive Gliosis Following Kainic Acid Injection into the Rat Striatum. *Neurosci Lett* **168:** 193–196.
36. Streit, W. J., and G. W. Kreutzberg. 1987. Lectin binding by resting and reactive microglia. *J. Neurocytol.* **16:** 249–260.
37. Streit, W. J., and G. W. Kreutzberg. 1988. Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. *J. Comp. Neurol.* **268:** 248–263.
38. Vela, J. M., I. Dalmau, L. Acarin, B. Gonzalez, and B. Castellano. 1995. Microglial cell reaction in the gray and white matter in spinal cords from jimpy mice. An enzyme histochemical study at the light and electron microscope level. *Brain Res.* **694:** 287–298.
39. Vela, J. M., I. Dalmau, B. Gonzalez, and B. Castellano. 1995. Morphology and distribution of microglial cells in the young and adult mouse cerebellum. *J. Comp. Neurol.* **361:** 602–616.
40. Villablanca, J. R., J. W. Burgess, and F. Benedetti. 1986. There is less thalamic degeneration in neonatal-lesioned than in adult-lesioned cats after cerebral hemispherectomy. *Brain Res.* **368:** 211–225.
41. Wu, C. H., C. Y. Wen, J. Y. Shieh, and E. A. Ling. 1992. A quantitative and morphometric study of the transformation of amoeboid microglia into ramified microglia in the developing corpus callosum in rats. *J. Anat.* **181:** 423–430.
42. Wu, C. H., C. Y. Wen, J. Y. Shieh, and E. A. Ling. 1993. A quantitative study of the differentiation of microglial cells in the developing cerebral cortex in rats. *J. Anat.* **182:** 403–413.
43. Xu, J., and E. A. Ling. 1994. Upregulation and induction of major histocompatibility complex class I and II antigens on microglial cells in early postnatal rat brain following intraperitoneal injections of recombinant interferon-gamma. *Neuroscience* **60:** 959–967.