Response of Macrophage/Microglial Cells to Experimental Neuronal Degeneration in the Avian Isthmo-optic Nucleus During Development

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ABSTRACT

Blockade of the retrograde axonal transport of isthmo-optic nucleus (ION) neurons in the avian embryo results in their massive degeneration. We used this system to investigate the response of macrophage/microglial cells to neuronal degeneration in the embryonic brain. Colchicine was injected into the right eye of quail or chick embryos at a time when the survival of ION neurons depends on retrograde trophic support from the retina, and the chronology of the subsequent macrophage/microglial response in the ION was analyzed. This response was restricted to the ION contralateral to the injected eye; no modifications of the normal state were observed in the surrounding parenchyma or in the opposite ION, used as control. The response was first detected 18 hours after the colchicine injection (18 hours pi), when an increase of the macrophage/microglial cell number was evident. The number of these cells in the affected ION increased, peaking at 40–48 hours pi. At later survival times, macrophage/microglial cells were progressively less abundant in the affected ION, which gradually diminished in size. At 120 hours pi the only remnant of the ION was a small cluster of macrophage/microglial cells, surrounded by a clear area with scarce nonmicroglial cells, in the region formerly occupied by the ION. This study reveals that a strong macrophage/microglial response occurs in the embryonic brain in response to neuronal degeneration but that these cells do not trigger the neuronal death, as they only appear after pyknotic fragments are already observable. J. Comp. Neurol. 423:659–669, 2000. © 2000 Wiley-Liss, Inc.
The isthmo-optic nucleus (ION) of birds is the source of retinopetal axons that connect the brain with the contralateral retina (Clarke, 1992). The cells forming the ION constitute a well-defined ovoid structure in the dorsal aspect of the meso-metencephalic border. Eye ablation or intracocular injection of excitotoxins or blockers of axonal transport induces the retrograde degeneration of nearly all neurons of the contralateral ION (Cowan and Wenger, 1968; Clarke et al., 1976; O'Leary and Cowan, 1984; Clarke, 1992). In particular, the intracocular injection of colchicine after the arrival in the retina of axons from the ION blocks retrograde transport along these axons, leading to degeneration of the ION neurons (Clarke, 1982a). In all these situations of retrograde degeneration, the ION contains numerous small cells that participate in the phagocytosis of cell debris. Although some of these cells appear to be autophagic dying neurons (Hornung et al., 1989; Clarke and Hornung, 1989), we show in the present paper that many are macrophages/microglia. Finally, when the degenerative phenomena have finished, the area formerly occupied by the ION is just distinguishable because of the presence of a small cluster of nonneuronal cells (Cowan and Wenger, 1968; O'Leary and Cowan, 1984).

In the present paper we study the response of macrophage/microglial cells within the degenerating ION after an injection of colchicine in the contralateral eye during embryonic development. We have determined the distribution of such cells at different times after the colchicine injection and compared it with the distribution of similar cells in the ipsilateral (nonaffected) ION and in the IONs of normal embryos. Our results show that the macrophage/microglial response is faster than that described in postnatal animals.

MATERIALS AND METHODS

Normal embryos

The study of microglial cell development in the normal ION was carried out on 23 quail embryos from incubation day 7 (E7) to E16, which is normally the last incubation day for the quail. Nine posthatching quails were also used; their age ranged from a few hours after hatching (P0) to P10. The brains of these animals were dissected, fixed in Bouin's fluid for 3–5 days, and embedded in paraffin. Posthatching quails were anesthetized with ether and perfused through the heart before decapitation and dissection of the brain. Transverse paraffin sections (10 µm thick) were immunolabeled with the QH1 antibody, revealed with peroxidase histochemistry, and counterstained with hematoxylin as described in previous articles (Cuadros et al., 1994, 1997). The QH1 antibody recognizes in the quail all developmental phases of macrophage/microglial cells (Cuadros et al., 1992) in addition to endothelial cells (Pardanaud et al., 1987). Besides these sections, sagittal sections of brains prepared previously (Cuadros et al., 1997) containing the IONs were also used during this study.

ION degeneration

Degeneration of the ION neurons was induced by injecting 0.4 µg of colchicine (Sigma, St. Louis, MO) in the right eye of E11 quail embryos. This age was selected for injections because the axons of ION neurons have reached the contralateral retina and started to form synapses well before this time (see Discussion), and their survival is acutely dependent on the receipt of retrograde trophic support from the retina. Injection of colchicine at a comparable stage of development in chick embryos induces massive degeneration of ION neurons beginning 18 hours post injection (18 hours pi; Primi and Clarke, 1997). After injection, the embryos were returned to the incubator for different times (summarized in Table 1), fixed in Bouin's fluid, and processed as described above for normal embryos.

Chick embryos were also used for comparison with the data obtained in quail embryos. They received an intracocular injection of colchicine (0.25–0.4 µg) at E12–E14 and were fixed in 4% paraformaldehyde after 35–40 hours of survival. Sections (20 µm thick) were obtained in a cryostat and immunostained with the antibody HIS-C7 (a generous gift from Dr. S.H. Jeurissen), which labels all types of chicken leukocytes (Jeurissen et al., 1988). To reveal the presence of pyknotic fragments in the ION, some immunostained sections were also labeled for 5 minutes in a solution containing 1 µg/ml of Hoechst 33342 (Sigma). The experimental procedures described above were in accordance with specifications from the Unit of Laboratory Animals of the University of Granada, Spain, and the regulations of the Canton de Vaud, Switzerland.

Quantitation of data

We determined the density of QH1+ cells in each ION using five sections passing through the central region. The area of the ION was measured every fifth section; the sum of these values multiplied by the section thickness (10 µm) and by the sampling interval (5) was considered to be the ION volume. Estimations of the total number of QH1+ cells per ION were obtained from QH1+ cell densities and ION volumes. This method overestimates the number of QH1+ cells in normal IONs, as slightly higher densities of these cells were observed in central than in rostral and caudal parts of the ION, but it provides an accurate estimation of the QH1+ cells number in affected IONs, where these cells were apparently distributed homogeneously. Because at 120 hours pi the ION remnant does not have clear borders and has greatly decreased in size, the number of QH1+ cells in them were counted in every section to obtain their total number.

To determine when experimental degeneration begins, pyknotic fragments were counted in IONs of both 12- and 18-hour pi quail embryos. Camera lucida drawings of representative sections of control and affected IONs were made at each time point; the locations of QH1+ cells were indicated on these drawings.
Terminology

The QH1 antibody labels not only macrophage/microglial cells in the quail, but endothelial cells also. To avoid the reiteration of expressions like “nonendothelial labeled cells” or “free QH1+ cells,” throughout this paper we shall use “QH1+ cells” to designate exclusively macrophage/microglial cells; if we wish to allude to the blood vessels or endothelial cells, we will refer to them explicitly.

RESULTS

Microglial development in the normal ION

The ION primordium can be detected as a part of the isthmic migration (Clarke, 1982b) in E8 quail embryos. No QH1+ cells different from endothelial cells were observed within it, although a few QH1+ cells were seen around the anlage of the ION (Fig. 1A). At E10, the convoluted sheet arrangement of the ION was first visible in some embryos. Even though several QH1+ blood vessels traverse the ION anlage, less than 30 QH1+ cells were seen within it (Fig. 1B).

The increase in volume and the development of the convoluted sheet arrangement of the ION in subsequent stages was accompanied by an increase of the number of QH1+ cells within it (around 100 QH1+ cells per ION at E12), nearly always located in regions rich in cell bodies (Fig. 1C). Many of the QH1+ cells were ameboid and located around vessels traversing the ION, whereas other QH1+ cells showed a primitive ramification (Fig. 1D,E). A conspicuous fact from E10 on was that QH1+ cells appeared at the edge of the ION, being more frequent at its ventral border (Fig. 1B–D); these cells were mostly ameboid at E10 but progressively became ramified. The general distribution of microglial cells within the ION did not change very much from E10 until hatching, although their number nearly doubled at the last incubation days. During these stages of development the proportion of QH1+ cells showing ramified morphology increased both within the ION and around it.

Two main changes were observed in the QH1+ cells of the P0 ION (Fig. 1F). First, there were very few, if any, ameboid cells. Second, QH1+ cells increased in number in neuropil regions of the ION, where few had been observable at the end of embryonic development. In the P10 ION, QH1+ cells showed longer and thinner processes than at P0–P2. The QH1+ cells in the P10 ION were present both in the neuropil and in regions rich in cell bodies, without any apparent restriction in their distribution (Fig. 1G,H).

Macrophage/microglial response in the ION after colchicine injection

The period of normal cell death of ION neurons occurs in chick embryos at E13–E17, and the ION’s formation of synaptic connections onto retinal neurons likewise begins at E13 (Clarke, 1992). In quail embryos, ION development is thought to occur about 4 days in advance of that of the chick (Martinez and Alvarado-Mallart, 1989), and this is confirmed by our observation that the convoluted sheet arrangement of the ION neurons became detectable at E10 or shortly after, whereas in the chick it appears at E14 (Clarke, 1992). Hence, in the present experiments, the day of injection (E11) corresponds to about E15 in the chick, which is near the middle of the ION’s period of neuronal death and 2 days after the onset of isthmo-retinal synaptogenesis. Hematoxylin staining of normal E11 quail embryos confirmed the presence of dying neurons in the ION.

Colchicine injections in one eye of quail embryos at E11 caused a massive degeneration in the contralateral ION. Comparable results were obtained when colchicine was injected into one eye of chick embryos of equivalent age and after similar survival times. The cell death processes occurring in the IONs of both quail and chick embryos were accompanied by a macrophage/microglial response restricted to this structure (Fig. 2A–C). This response apparently did not affect the presence or the features of microglial cells in the surrounding nervous tissue. The only other region showing a clearly affected pattern of distribution of macrophage/microglial cells was located under the pial surface of the optic tectum contralateral to the injection, where these cells concentrate, apparently in response to the degeneration of the optic axons coursing through this area (Fig. 2D,E).

As similar results were found in chick and quail embryos, we chose to focus our study on this latter species largely for reasons of technical convenience.

In the embryos analyzed at 12 hours pi, no clear differences in the number (Fig. 3) or distribution of QH1+ cells were observed between the control and affected IONs. Similar numbers of pyknotic nuclei, as revealed by the hematoxylin counterstain, were present within the affected and control IONs. At 18 hours pi, however, many more pyknotic fragments were seen in the affected ION than in the control one (Table 2). Coincidently, more QH1+ cells appeared in the affected than in the control ION (Figs. 3, 4A, 5). It is noteworthy that the increase in numbers of macrophages/microglia in the affected ION was somewhat variable between embryos but was always greater in anterior than in posterior regions of the ION. The anterior part of the affected ION showed an increase in the number of QH1+ cells, but lower numbers of QH1+ cells were present in the posterior ION, despite the fact that pyknotic nuclei were frequent there. Most of the QH1+ cells in the affected ION showed a primitive ramification or short and broad processes; some round cells were also present, chiefly in the rostral part.

At 24 hours pi more QH1+ cells appeared within the affected ION than at 18 hours pi, and their number increased again at 30 hours pi (Figs. 3, 5). At 30 hours pi QH1+ cells occupied most of the volume of the affected ION (Figs. 4B, 5), and the proportion of round QH1+ cells was higher than at the previous time point. The number of QH1+ cells in the affected ION reached its maximum at 40–48 hours pi (Figs. 3, 4C, 5), when they showed variable morphologies (Fig. 4D–F). Pyknotic fragments were sometimes seen within or in contact with QH1+ cells, strongly suggesting that the QH1+ cells engulfed dead cell fragments (Fig. 4F). This was confirmed by Hoechst-anti HIS-C7 double labeling experiments performed in chick embryos (Fig. 5).

At 72 hours pi the remnant of the affected ION was reduced in size (Fig. 4G). The QH1+ cells occupied most of it (Figs. 4G, 5), but their total number was lower than at 40 and 48 hours pi (Fig. 3). The surrounding parenchyma, corresponding to the area occupied by the ION in normal embryos, had an “empty” appearance, as it contained very few cells. The QH1+ cells in the affected ION were continuous with a “stream” of labeled cells appearing among
Fig. 1. Distribution of QH1+ cells during development of the normal quail ION. A: At E8, only endothelial cells of blood vessels (open arrows) are labeled by QH1 antibody in the region where the primordium of the ION develops. B: A group of ameboid QH1+ cells (arrows) are seen in the central region of an E10 ION (delimited by asterisks). C: Ameboid cells (arrows) are labeled, in addition to blood vessels, within an E13 ION. D: Parasagittal section of an E12 brain, showing the left ION. The boxed area appears in E at higher magnification. Several QH1+ cells (arrows) are seen delimiting the border of the ION. E: Higher magnification of the boxed area in D, showing two QH1+ cells in contact with blood vessels (open arrows); one of them (arrowhead) has a round morphology, whereas another (arrow) displays several processes. F: Ramified QH1+ cells (arrows) in the ION at the day of hatching (P0). G,H: Ramified QH1+ cells (arrows) in P10 embryos, in both cellular and neuropil regions of the ION. Isolated processes of microglial cells whose cell bodies do not appear in the picture are also seen. Scale bar = 75 μm in A; 130 μm in B,D; 90 μm in C; 30 μm in E; 70 μm in F; 40 μm in G,H.
the fibrous tissue that connects the isthmic region to the ipsilateral optic lobe.

At 96 hours pi, the QH1+ cells in the affected ION occupied a still smaller region, and their total number was lower than at 72 hours pi (Figs. 3, 5).

The last time point analyzed, 120 hours pi, corresponded to E16 embryos. In these the only remnant of the affected ION was a small cluster of QH1+ ameboid cells (Fig. 4H) located underneath the pial border and/or around a large vessel coursing parallel to this border. The “empty” area around the ION remnant was larger than at previous time points. These observations match previous reports that the ION contralateral to an extirpated optic vesicle disappears before hatching (Cowan and Wenger, 1968; O'Leary and Cowan, 1984).

**DISCUSSION**

Our results document the normal pattern of microglial development in the avian ION and reveal that a rapid
A macrophage/microglial cell reaction is elicited by experimental neuronal degeneration during embryonic development. The rapidity of this reaction distinguishes it from reactions observed in the postnatal brain.

**Development of microglia in the normal ION**

Relatively small numbers of microglial cells can be seen in the avian ION during normal development, despite the cell death occurring during normal development, which affects more than 50% of the neurons present originally in the ION (Clarke, 1992). In view of these small numbers, and the lack of any striking change in microglia during the neuronal death period (probably E9–E13 in quails), the question arises of whether the immature microglial cells play a role in other developmental processes, such as the morphological organization of the developing nucleus, or whether they are just colonizing a particular region of the nervous parenchyma where they subsequently will differentiate (Cuadros and Navascués, 1998). Our observations in hatched quails confirm that the QH1+ cells seen in the embryonic IONs become ramified microglia. The sources of the QH1+ cells were not clear. Microglial cells were seen frequently around blood vessels, suggesting that they enter the nervous parenchyma from the bloodstream. A "stream" of QH1+ cells, considered to be microglial cells in the process of migration along the nervous parenchyma (Cuadros and Navascués, 1998), are present in the medial border of the optic lobe during most of the embryonic period; this stream continues toward the developing ION, and therefore may also be a source of microglial precursors for the ION.

**Macrophage/microglial response after massive cell death**

Neuronal degeneration in the ION induced by intraocular colchicine injection has several advantages for studying the response of macrophage/microglial cells to cell degeneration in the embryo. First, the injury is produced in the eye, far away from the ION, and therefore there is no direct injury of the ION disturbing the normal organization of the parenchyma and blood vessels. We can thus exclude extraneous factors added to the retrograde degeneration of ION neurons, which might influence the glial

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**TABLE 2. Mean Number of Pyknotic Fragments per Section Between Affected and Control IONs That Survived 12 and 18 Hours After an Intraocular Colchicine Injection at E11**

<table>
<thead>
<tr>
<th>Survival time (hr)</th>
<th>Control ION</th>
<th>Affected ION</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>3.4</td>
<td>4.0</td>
</tr>
<tr>
<td>12</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>18</td>
<td>3.4</td>
<td>21.4</td>
</tr>
<tr>
<td>18</td>
<td>3.8</td>
<td>49.4</td>
</tr>
</tbody>
</table>

Errors for the un.injected embryos: n = 2 for E13; n = 4 for E15 and E16; n = 6 for E12 and E14. For the colchicine-injected embryos: n = 3 for E11+30, E11+72, and E11+96; n = 4 for E11+48; n = 2 for other time points.
response. Second, the induced degeneration is limited to one ION, whereas the surrounding parenchyma and the contralateral ION appear normal; this provides the possibility of comparing the experimental ION with control regions in the same section.

Morphological criteria alone would not have been sufficient for identifying the macrophage/microglial cells, because there appear to be two kinds of vacuolated cell in the degenerating ION in our experimental model. Studies combining morphological criteria, horseradish peroxidase...
inoculation, and thymidine autoradiography after intraocular colchicine injection in the chick embryo indicated that many highly vacuolated cells containing debris in the affected IONs were neurons undergoing autophagic-type cell death (Clarke and Hornung, 1989; Hornung et al., 1989). Our present observations demonstrate that numerous nonneuronal phagocytes, almost certainly macrophages or microglial cells, remove cell debris in the affected ION. Our identification of these cells is strongly supported by the fact that we obtained similar results in quail and chick with two different antibodies, both specific for a subclass of hematopoietic cells (and endothelial cells in the case of QH1).

As early as 18 hours after the colchicine injection, the number of macrophages/microglial cells increases in the affected ION, and it continues to rise until about 48 hours after the injection. The macrophage/microglial cells, which generally are round ameboid cells or show some short processes, cluster in the specific region where the cell degeneration occurs. QH1+ cells in neighboring regions of the nervous parenchyma seem not to be affected, as no variations in the number and morphological features of QH1+ cells are noticeable in these regions with regard to the controls, despite the probable degeneration in these areas of so-called ectopic isthmo-optic neurons, which in chick embryos degenerate following an E15 intraocular injection of colchicine (Catsicas and Clarke, 1987).

The delay in the variation of the normal QH1+ cell pattern in the affected ION apparently corresponds to the time passing between the colchicine injection and the beginning of massive degeneration in the ION contralateral, which took place 12–18 hours after the injection in our quail embryos (Table 2), as also in the chick embryo (Primi and Clarke, 1997). Therefore, the first signs of a macrophage/microglial response in our system starts less than 6 hours after the cellular degeneration in the ION. This is in contrast to several studies reporting that macrophage/microglial cells show little alteration before 24 hours (Milligan et al., 1991b; Morioka and Streit, 1991; Wu et al., 1997; Graeber et al., 1998) or several days (Lawson and Perry, 1995) after a perinatal brain injury in the rat. However, in one study study in P6 rats, a microglial reaction did occur within 10 hours after an injection of N-methyl-D-aspartate (Acarin et al., 1996).

Origin and nature of macrophage/microglial cells responding in the developing brain

The origin of the new macrophage/microglial cells is not clear; it might be attributed to a) entrance of QH1+ cells from the blood stream, traversing the blood vessel endothelium; b) migration of QH1+ cells from the meninges, traversing the pial surface of the brain; c) migration of QH1+ cells from other regions of the brain parenchyma; and/or d) local proliferation of QH1+ cells already present. Our morphological observations are compatible with possibilities a and b. The arrival of macrophage/microglial cells coming from the parenchyma around the affected ION is unlikely to be the source of a significant proportion of labeled cells in the ION, as no evident alterations in the density and morphology of QH1+ cells were observed in these areas at any of the postinjection times examined. We have not observed enough QH1+ or HIS-C7+ mitotic cells to support option d in spite of the presence of frequent nonlabeled mitotic cells; more detailed studies are need to ascertain this point.

In connection with this latter possibility, thymidine autoradiographic studies in the chick ION after intraocular injection of colchicine indicated that phagocytic cells rarely if ever went into S-phase near the time of neuronal death (Clarke and Hornung, 1989), and apparently the proliferative capability of microglial cells during their response to brain injury in the newborn rat is lower than in the adult (Graeber et al., 1998). It is conceivable that all, or several, of these mechanisms may be involved in the increase of QH1+ cell number, but the importance of each one remains to be established.

Fig. 5. Camera lucida drawings from the central region of control and affected IONs at different times after colchicine injection at E11. Each dot represents a QH1+ cell. The number of hours of embryo survival after injection are indicated after the “+” symbol. Scale bar = 200 μm.
The nature of the phagocytic cells participating in the response to a CNS damage remains controversial (Riva-Depaty et al., 1994; Amat et al., 1996). It is currently accepted that the first macrophagic cells responding to an injury in the adult brain are microglia already present in the nervous parenchyma and that immigrating macrophages derived from circulating blood monocytes appear in later stages of the response (Amat et al., 1996; Schroeter et al., 1997). The case of an injury to the immature brain is quite different. Milligan et al. (1991b) concluded that the cells responding to cell death induced by a lesion in the perinatal rat brain are predominantly brain macrophages, presumably of blood origin, whereas microglial cells were apparently not involved. On the other hand, the cells responding to a similar injury in the adult brain are mainly microglial cells (Milligan et al., 1991b). Graeber et al. (1998) also observed that the macrophage/microglial response in the newborn rat brain is linked to the presence of large numbers of macrophages, which are not seen in the adult response; in addition, they remark that no infiltration of the affected area of the perinatal brain by blood-derived mononuclear cells is observable, implying that these cells derive from intrinsic microglia.

In any case, macrophages of blood origin would seem inherently more likely to be involved in the response to a lesion in the immature brain, before the blood-brain barrier is completely formed: several days after hatching in birds (Stewart and Wiley, 1981; Ribatti et al., 1993) and almost 2 weeks after birth in the rat (Xu et al., 1993). The antibodies used in this study label both microglial and macrophagic cells and therefore did not enable us to determine whether the cells responding to neuronal degeneration in the embryo were already committed to become microglia or whether they were recruited into the CNS in response to the injury.

**Final fate of the macrophage/microglial cells**

The macrophage/microglial cells that accumulate in the ION in response to cell degeneration disappear progressively, and 5 days after the injection only a small cluster of cells can be seen in the region previously occupied by the ION. Several explanations can be proposed to explain this fact. The first is that macrophage/microglial cells leave the region after fulfilling their function. The QH1+ cells seen at 120 hours pi are concentrated around a large blood vessel, which might be used by the macrophage/microglial cells to evacuate the area. An alternative explanation is that they may degenerate in situ; at present we do not have evidence of death of ION macrophage/microglial cells, but such a phenomenon has been reported in the adult rat (Gehrmann and Banati, 1995). A few of the macrophage/microglial cells might differentiate in situ, becoming ramified microglia; however, it is clear that most of them leave the area or die, since the number of QH1+
cells decreases by about 75% between 2 and 5 days after the colchicine injection.

**Relationship between cell death and macrophage/microglial cell response**

It is clear that QH1+ cells concentrate in the affected ION after the onset of its degeneration and are involved in the phagocytosis of cell debris, as in many other systems (Perry and Gordon, 1991; Pearson et al., 1993; Streit 1996; Chaudhary et al., 1999). QH1+ cells may be attracted toward the degeneration zone by factors released during cell death. These factors have not been identified, but some molecules, such as the monocyte chemoattractant protein-1, are candidates for involvement of phenomena of brain macrophage recruitment in normal development and after injury (Mallat et al., 1996; Ivacko et al., 1997; Cuadros and Navascués, 1998). However, it has also been observed that macrophage/microglial cells are absent from some regions where cell death is occurring during development (Milligan et al., 1991a; Rakic and Zeccevic, 1998), including the external granule layer of the cerebellum (Cuadros et al., 1997) and the internal nuclear layer of the embryonic avian retina (Marín-Teva et al., 1999). Also, only small numbers of macrophage/microglial cells arrive in the ION during normal development in spite of the death of numerous neurons (this paper).

Likewise, we could detect no macrophage/microglia response in the optic tectum (except in the superficial stratum opticum; compare Fig. 2D and E), although substantial cell death does occur after an intraocular colchicine injection, both in the chick (Catsicas et al., 1992) and in the present quail embryos (unpublished observations). Perhaps attractant factors are not released in some cell death phenomena, which would explain the lack of macrophage/microglial cell response, or perhaps the amount released is below a critical level needed for eliciting a major macrophage/microglial response.

The presence of macrophage/microglial cells in the area before the beginning of neuronal degeneration may also influence the recruitment of new cells. In this connection, Caggiano and Brunjes (1993) showed that enhancement of cell death during development of the olfactory bulb apparently does not affect the normal distribution pattern of microglial cells within this structure; these authors suggested that the high number of microglial cells present in the normal olfactory bulb may be sufficient to remove the increased cell debris without the involvement of new macrophagic cells (Caggiano and Brunjes, 1993). In this view, an increase of macrophage/microglial cells would be expected in response to enhanced degeneration in regions, like the ION, with a rather low basal density of microglia, where the increased cell debris would require a corresponding increase in phagocytic capacity.

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**LITERATURE CITED**


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