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Immunocytochemical demonstration of astrocytes and microglia in the whale brain

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Whale brains have attracted the attention of neuroscientists but there are only sparse studies on whale glial cells. Here we report on immunolabeling of astrocytes by antibodies to glial fibrillary acidic protein (GFAP) or protein S-100β (both by the streptavidin/biotin technique), and labeling of microglial cells by *Griffonia simplicifolia* agglutinin (GSA I-B_4, coupled to horseradish peroxidase), in the neocortex of a harbour porpoise *Phocoena phocoena* L. Many subpial and perivascular astrocytes were stained; they differed greatly in thickness and length of their processes. Subpial astrocytes were coarse with a few stout stem processes, whereas perivascular astrocytes deeper in the brain had many long and slender processes. Additionally, some long radial astrocytes were observed. Microglia were labeled throughout the brain, and showed similar features as 'resting' (ramified) microglia in the brain of other mammals.

Whereas over the years sound data have accumulated on both the gross anatomy and the neuronal cells of the brains of *Cetacea* including whales and dolphins [6], there is only very sparse information on cetacean neuroglia. It has been reported that the whale brain is characterized by a high density of astroglia-like cells [2], and by a high glia:neuron index [2,3]. The ultrastructure of the astroglia-like cells, as well as their relation to blood vessels, has been described, and some Golgi-impregnated astrocyte-like cells have been shown [2]. It has been pointed out that dolphine glial cells show features of both astrocytes (many intermediate filaments) and oligodendrocytes (ultrastructure of the nucleus, and cytoplasmic organelles) [2]. The authors conclude that 'to determine the actual character of these morphologically intermediate cells, it would be extremely important to apply the immunofluorescence reaction to GFAP (i.e. glial fibrillary acidic protein) which is specific for astrocyte(s)...' [2]. To the best of our knowledge, this has not yet been done. Likewise, nothing is known on the structure of microglial cells in whale brains. This prompted us to perform immunolabeling of astrocytes and histochemical demonstration of microglial cells in the brain of a harbour porpoise.

A male harbour porpoise (*Phocoena phocoena* L.) captured by accident in the North Sea (Heiligenhafen) was offered for scientific studies. Six hours after its death (caused by severe injury due to the ship's crew), the brain was isolated and fixed in formaline (Fig. 1A). A tissue block from motor neocortex was paraffin embedded and sections were cut of a thickness of 20 μm. A series of the sections was stained by Cresyl violet. In some sections, lectin histochemical visualization of microglial cells [12] was performed. *Griffonia (Bandeiraea) simplicifolia* agglutinin (GSA I-B_4) coupled to HRP was obtained from Sigma (L-5391; St. Louis, MO); sections were incubated free-floating overnight at 4°C with the lectin diluted to 20 μg/ml in PBS containing cations and 0.1% Triton X-100. After three washes, lectin binding sites were localized using 3,3′-diaminobenzidine (DAB)/H_2O_2.

Another series of sections was used for immunocytochemistry. We used an antiserum directed against S-100β [2] (IgG fraction, 1:1000 to 1:3000; East Acres, Southbridge, MA) which was found to be specific for glial cells [9]. GFAP was labeled with rabbit anti-cow GFAP antiserum (1:1000 to 1:3000; DAKO, Copenhagen, Denmark) [19]. For the detection of both astroglial markers we applied a streptavidin/biotin technique, and DAB as

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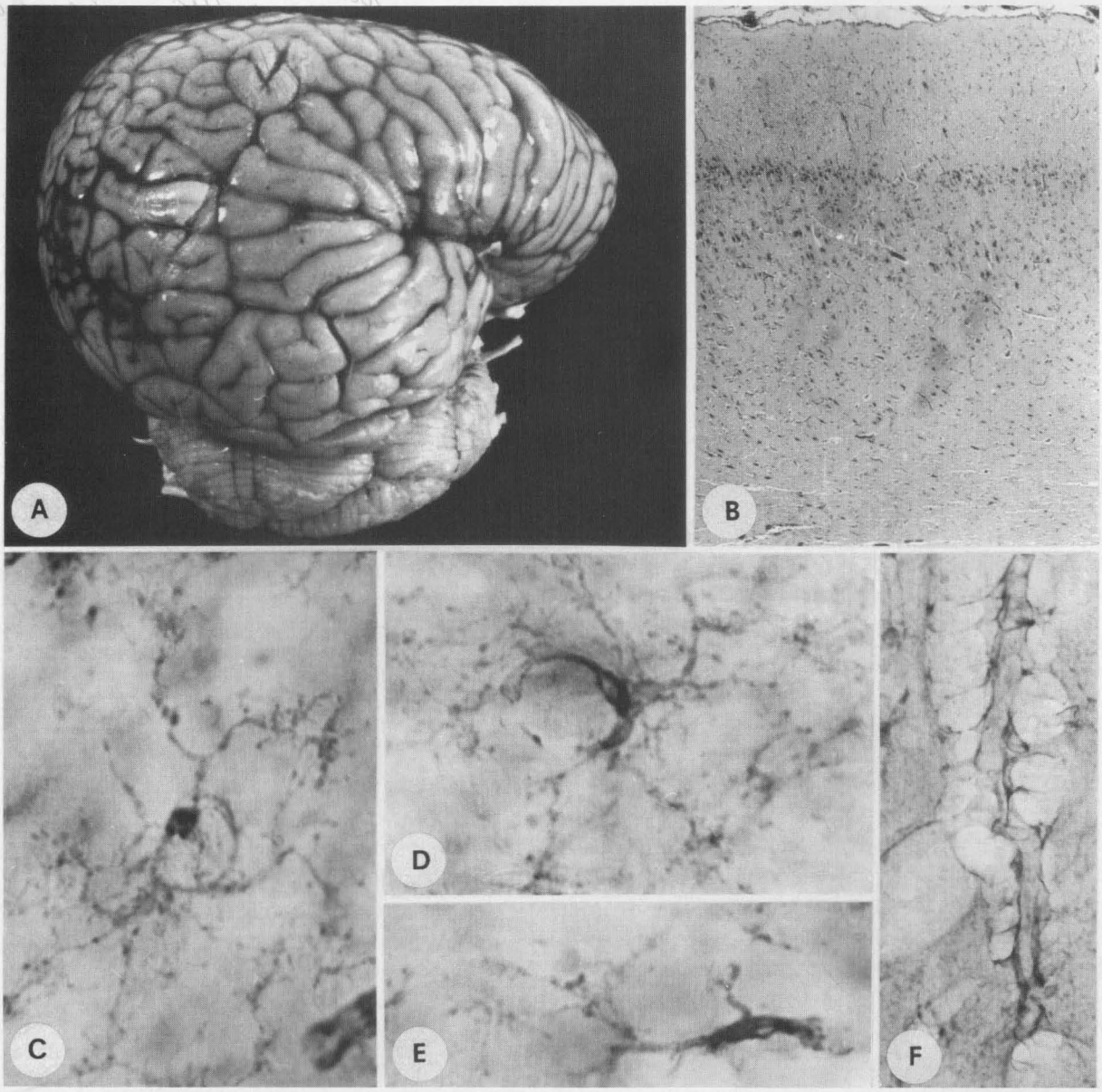

Fig. 1. The harbour porpoise brain. A: postero-dorsal view of the isolated brain; $^1/_4$ of original size. B: Cresyl violet stained section through the motor neocortex; × 30. C,D,E: microglial cells in the harbour porpoise brain. *G. simplicifolia* agglutinin GSA I-B_4 histochemistry; × 700. F: perivascular astrocyte endfeet labeled by S-100β immunocytochemistry; × 700.

chromogen. Controls were performed by omitting the primary antibodies.

Due to the non-optimal fixation (see above), the tissue showed local differences in accessibility to immunostaining although the gross histology was normal (Fig. 1B). Microglial cells were labeled throughout the sections by applying lectin cytochemistry. They were rather densely and regularly arranged, and were found in the form of typical 'resting' or arborized microglia (Fig. 1B,C,D).

Astrocytes were immunocytochemically labeled by two markers. Antibodies directed against S-100β stained cell bodies and proximal stem processes, as well as perivascular and subpial endfeet (Fig. 1F). By applying the GFAP antiserum, mainly subpial astrocytes were labeled (Fig. 2A–D). At some places, radially oriented long GFAP-positive processes were observed (Fig. 2A). Some of the cells had shorter but radially aligned processes (Fig. 2B) running towards the pia. Mostly, however,

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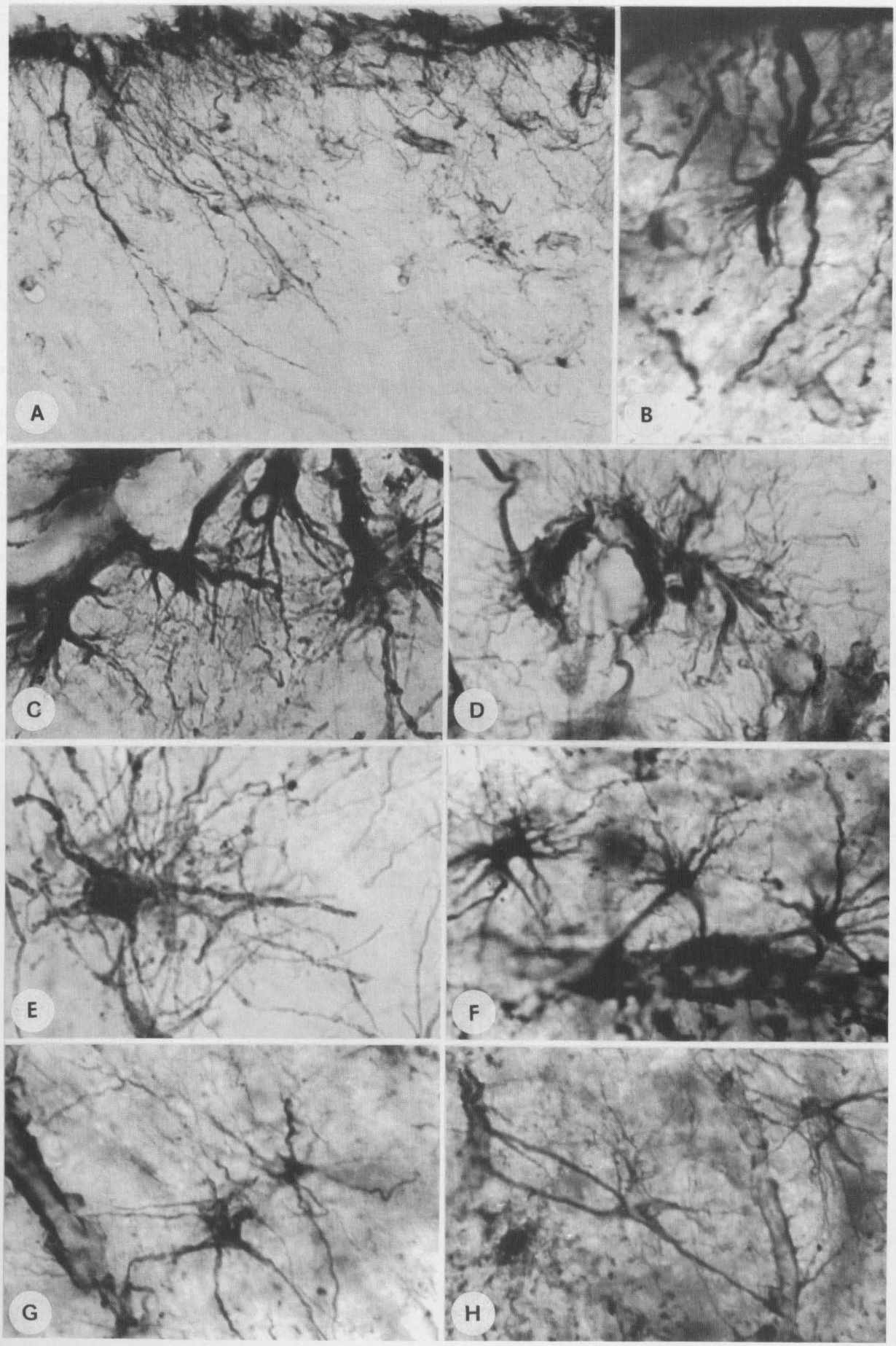
Fig. 2. Astrocytes of the porpoise brain labeled by GFAP immunocytochemistry. A: view on subpial astroglia at low magnification. The pia is at the top; × 180. B–D: coarse subpial astrocytes; the pia is always at the top. × 700. E–H: astrocytes in deeper layers, with perivascular endfeet; × 700.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subpial astrocytes were coarse with a few thick and short stem processes, and many short and thin end branches (Fig. 2C,D). Perivascular astrocytes deeper in the brain were typically more delicate, with many long and slender processes (Fig. 2E–H). All blood vessels were surrounded by a densely labeled sheeth of astrocytic endfeet (Fig. 2F–H). In some cases, one of these cells sent processes which formed endfeet at two and more small blood vessels (Fig. 2H).

This paper demonstrates, for the first time, cytochemical staining of microglial cells and immunocytochemistry of astrocytes of the whale brain. Porpoise microglial cells were labeled by the *Griffonia simplicifolia* agglutinin, as it had been shown for another aquatic mammal, the manatee [13], as well as for the rat [12]. As the pathology of the whale brain and glia is evolving [4], detailed knowledge of the normal glial cell morphology becomes important. In particular, it would be interesting to find out what morphological changes undergo microglial cells, activated in cases of morbillivirus infection [4].

Three main types of astrocytes were discernible; (1) radial astrocytes with long stout processes to the pia, (2) coarse subpial astrocytes, with large somata and few thick and short processes abutting the pia, and (3) delicate astrocytes with long thin perivascular processes. This equals the case found in the brain of most mammals [9] with the exception of long radial astrocytes, which are usually observed only in the early postnatal neocortex, or in the brain of lower vertebrates [9]. It has been pointed out that from a histological point of view, the whale neocortex is rather thin and simple when compared to the neocortex of primates and other higher mammals [7]. This might allow for a delay and even incompleteness of the transformation of radial glia into stellate astroglia, which normally occurs in the postnal period [8,9,14]. Anyhow, many cells showed dense labeling for GFAP, and may thus be considered as 'true' astrocytes [1]. GFAP-positive astrocytes with oligodendrocyte-like nuclear ultrastructure and organelle-rich cytoplasm [2] have been found not only in the Cetacean brain but also in the rabbit retina [10,11]. It remains an open question what specific functional task(s) may require this atypical ultrastructure in these cases.

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