Structure of the Cerebral Cortex of the Humpback Whale, *Megaptera novaeangliae* (Cetacea, Mysticeti, Balaenopteridae)

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ABSTRACT

Cetaceans diverged from terrestrial mammals between 50 and 60 million years ago and acquired, during their adaptation to a fully aquatic milieu, many derived features, including echolocation (in odontocetes), remarkable auditory and communicative abilities, as well as a complex social organization. Whereas brain structure has been documented in detail in some odontocetes, few reports exist on its organization in mysticetes. We studied the cerebral cortex of the humpback whale (*Megaptera novaeangliae*) in comparison to another balaenopterid, the fin whale, and representative odontocetes. We observed several differences between *Megaptera* and odontocetes, such as a highly clustered organization of layer II over the occipital and inferotemporal neocortex, whereas such pattern is restricted to the ventral insula in odontocetes. A striking observation in *Megaptera* was the presence in layer V of the anterior cingulate, anterior insular, and frontopolar cortices of large spindle cells, similar in morphology and distribution to those described in hominids, suggesting a case of parallel evolution. They were also observed in the fin whale and the largest odontocetes, but not in species with smaller brains or body size. The hippocampal formation, unremarkable in odontocetes, is further diminutive in *Megaptera*, contrasting with terrestrial mammals. As in odontocetes, clear cytoarchitectural patterns exist in the neocortex of *Megaptera*, making it possible to define many cortical domains. These observations demonstrate that *Megaptera* differs from Odontoceti in certain aspects of cortical cytoarchitecture and may provide a neuromorphologic basis for functional and behavioral differences between the suborders as well as a reflection of their divergent evolution. Anat Rec, 290:1–31, 2007.

Key words: balaenopterids; cetaceans; cytoarchitecture; dolphins; mysticete; neocortex; odontocete

The order Cetacea comprises highly diversified species distributed into two suborders, the Mysticeti (baleen whales) and the Odontoceti (toothed whales). The suborder Mysticeti includes 13 species (the so-called right whales, the pygmy right whale, the gray whale, and the rorquals), while Odontoceti contains 72 species of toothed whales, dolphins, and porpoises, distributed into four superfamilies (Mann et al., 2000). Morphological features and molecular phylogenetics suggest that cetaceans are directly related to artiodactyls (even-toed ungulates) (Gingerich et al., 2001; Thewissen et al.,

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2001; Geisler and Uhen, 2003). In fact, a sister-group relationship exists between modern cetaceans and hippopotamids (Nikaido et al., 1996; Shimamura et al., 1997; Gatesy, 1998; Milinkovitch et al., 1998), even though they diverged about 52 million years ago (Gingerich and Uhen, 1998). The fossil record indicates that the earliest cetaceans, the Archaeoceti, appeared in Indo-Pakistan about 55 million years ago and survived until approximately 38 million years ago in the late Eocene (Barnes et al., 1985; Carroll, 1988; Fordyce and Barnes, 1994; Thewissen et al., 1996; Thewissen and Williams, 2002; Geisler and Sanders, 2003). The modern suborders appeared and began to diverge in the early Oligocene around that same time (Barnes et al., 1985; Carroll, 1988; Fordyce and Barnes, 1994). The earliest baleen whale fossils are found in the early Oligocene of Antarctica and late Oligocene of North America (Emlong, 1966; Fordyce, 1977, 2002; Fordyce and Barnes, 1994; Geisler and Sanders, 2003; Fitzgerald, 2006), yet the earliest mysticetes were not likely to be filter feeders but have been shown to be macrophagous animals (Fitzgerald, 2006). The oldest fossil considered to represent a member of the genus *Megaptera* is known from the Late Miocene of California (*M. miocaena*, 6 million years ago) (Kellogg, 1922), although its position is debated (Deméré et al., 2005). More recent fossils have been tentatively identified from the Late Pliocene and Pleistocene of North America and Europe (Romer, 1966; Deméré et al., 2005). Whereas the biology of the humpback whale is well documented (for review, see Clapham and Mead, 1999; Clapham, 2000), there is virtually no information in the literature on the structure of its brain beyond isolated descriptions of generic surface features (Breathnach, 1955; Pilleri, 1966b, 1966c), and as such, possible relationships between the extensive repertory of behavioral and social abilities, including the complexity of vocal productions, and brain morphologic specializations remain unknown for this species, as well as for other mysticetes.

The brains of extant cetaceans are among the largest in absolute size and in relation to body size of all mammals, the largest brain on earth being that of the sperm whale (8 kg on average) (Jerison, 1973; Marino, 1998, 2002a; Marino et al., 2004). Furthermore, many odontocetes have very high encephalization quotients compared to terrestrial mammals, close to the values of modern humans and higher than those of nonhuman anthropoid primates (Marino, 1998; Marino et al., 2004). Whereas the encephalization quotient of mysticetes is much lower compared to odontocetes (Marino, 2002a), the large absolute size, extensive cortical gyrification, and highly derived morphology of the mysticete brain suggest that it too was subjected to considerable expansion and elaboration during its evolution (Oelschläger and Oelschläger, 2002).

The regional organization of the cerebral cortex in cetaceans remains poorly understood, most of our knowledge of it stemming from studies of the bottlenose dolphin (Kesarev, 1969; Kesarev and Malofeeva, 1969; Jacobs et al., 1971, 1979, 1984; Kesarev et al., 1977; Morgane et al., 1982, 1988; Garey et al., 1985; Manger et al., 1998). In general, the dolphin neocortex is thin and agranular, harbors a very prominent thick layer I that is far more cellular than in terrestrial species, has large inverted neurons in the cell-dense layer II, and very large pyramidal neurons frequently forming clusters at the border between layers III and V. Layers III and VI vary considerably in thickness and cellular density across regions (Hof et al., 2005). In continuation of our investigations of cortical diversity in cetaceans, we had the opportunity to study the brain of a humpback whale in comparison with one other balaenopterid, the fin whale (*Balaenoptera physalus*), and a series of specimens including representative members of all of the major odontocete families (i.e., sperm whales, beaked whales, fresh water and estuarine dolphins, and delphinoids, including porpoises). We describe the general cytoarchitecture of the neocortex and hippocampal formation of *M. novaeangliae* and summarize regional differences with odontocetes whenever possible, in an attempt to reveal organizational features that may serve as correlates of functional specializations characterizing each of the two cetacean suborders. This report also constitutes a case study of cortical organization in baleen whales, inasmuch as *M. novaeangliae* can be considered representative of Mysticeti as a whole.

MATERIALS AND METHODS

The brain of a stranded adult female humpback whale (*Megaptera* novaeangliae, ~13.8 m, beak-to-fluke notch length) was extracted on site and postfixed in 8% formalin for at least 3 months. The left hemisphere, whose surface had been damaged during removal, was preserved whole in formalin. The right hemisphere was dehydrated in graded alcohol solutions, embedded in celloidin, and processed serially at 35 μm on a modified large specimen microtome (Mico Instruments, Cambridge, MA) (Jacobs et al., 1971, 1979, 1984). This hemisphere was cut in the sagittal plane relative to the beak-fluke axis of the animal. Two 1:20 series of adjacent sections throughout this hemisphere were stained alternatively for Nissl substance with the Bielchowsky-Plien cresyl violet method or for myelin with the Loyez-Weigert method (Bertrand, 1930). The sections were mounted on large glass slides and coverslipped in clarite for examination.

The brain of a female fin whale (*Balaenoptera physalus*, ~19 m) that was collected from a stranded animal was available for comparison. Only surface sampling of discrete cortical regions was performed on this intact specimen for comparison with the humpback whale specimen. The brains of three adult male bottlenose dolphins (*Tursiops truncatus*) were analyzed in detail to compare their cortical organization with that of the two balaenopterids. These three dolphins had been perfused by gravity with 40 l of Windle's fluid in situ using a cannula inserted into the descending aorta after they had been euthanized for medical reasons (Jacobs et al., 1971, 1979, 1984; Morgane et al., 1980, 1982). These brains were each sectioned in one of the three planes (i.e., coronal, sagittal, and horizontal to the beak-fluke axis) and stained in a 1:5 series, as described above for the humpback whale (Jacobs et al., 1971, 1979, 1984; Morgane et al., 1980, 1982). Adult specimens from an additional *T. truncatus* and from a sperm whale (*Physeter macrocephalus*), a dwarf sperm whale (*Kogia simus*), a Cuvier's beaked whale (*Ziphius cavirostris*), an Amazon river dolphin (*Inia geoffrensis*), a tucuxi (*Sotalia fluviatilis*), two beluga whales (*Delphinapterus leucas*), a long-finned pilot whale (*Globicephala melas*), a striped dolphin (*Stenella coeruleoalba*), a killer whale (*Orcinus orca*), a

Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), and a harbor porpoise (*Phocoena phocoena*) were used for comparison as representative odontocetes. These specimens were all obtained from stranded animals within a few hours of death and were fixed by immersion in neutral formalin for several months. The brains of *Stenella* and *Phocoena* were prepared in alternate 1:5 coronal series as described for the perfused *Tursiops* specimens. Local samples of cortical regions were obtained from all of the other cases, cryoprotected in graded sucrose solutions, cut on a cryostat (Reichert Jung, Vienna, Austria) at 60 or 100 μm thickness depending on the specimen. Series of sections were then stained with cresyl violet (1%; Sigma-Aldrich, St. Louis, MO) according to a standard protocol. Finally, sections from the human cingulate cortex were used to compare spindle cell morphology. These were obtained from control postmortem materials and processed as described in our previous studies of these neurons (Nimchinsky et al., 1995, 1999).

All histological preparations were examined and photographed on a Zeiss Axiophot 2 photomicroscope with 2.5×, 5×, 10×, and 20× Fluar and Apochromat objectives (Zeiss, Oberkochen, Germany). Photomicrographs were acquired using an Optronics MicroFire digital camera (Optronics, Goleta, CA). Photomontages were digitally assembled with VirtualSlice software (MicroBrightField, Williston, VT) and processed with Adobe Photoshop CS2. Macrographic overviews of the large histologic preparations of *Megaptera* and *Tursiops* were produced by scanning the glass-mounted sections on a flat-bed scanner. The resulting pictures were then manually edited in Adobe Photoshop. The nomenclature of gyri and sulci was adapted from Morgane et al. (1980) for odontocetes and *B. physalus*, and from Pilleri (1966b) for *M. novaeangliae*.

RESULTS

General Morphology and Histology

The fixed brain of the humpback whale weighed about 4.6 kg and measured 22.4 cm in its anteroposterior axis and 18 cm at the largest width of the temporal lobes (Fig. 1A and B). In comparison, the fixed brain of the fin whale was slightly larger, weighing about 5.2 kg for 25 cm in its anteroposterior axis and 28 cm of maximal lateral extent (Fig. 1C–F). Both specimens showed comparable surface morphology in the number and distribution of gyri and sulci. The medial aspect of the hemisphere of both balaenopterids showed a well-developed cingulate and retrosplenial cortex (the "limbic" lobe of Morgane et al., 1980), located below the splenial fissure (or limbic cleft) and containing two intercalate sulci in its anterior segment (Figs. 1E and 2A). The upper aspect of the midline cortex showed a deep paralimbic fissure [entolateral sulcus (Guldberg, 1885); sulcus confinis (Kükenthal and Ziehen, 1889; Pilleri, 1966b)] running from the inferomedial side of the temporo-occipital cortex toward the apex of the brain (Figs. 1E and 2A) and dividing the paralimbic lobe from the supralimbic lobe (Morgane et al., 1980). The lateral aspect of both hemispheres was characterized by a verticalized Sylvian fissure and a series of concentrically organized sulci defining the principal gyri of the temporoparietal operculum (Figs. 1B and F and 2B). The term "Sylvian," while established in the literature on cetacean brain, is not used here to convey homology with the Sylvian fissure of primates; it corresponds to the "pseudosylvian" fissure of carnivores. Immediately above the Sylvian fissure, separating the sylvian gyrus from the ectosylvian gyrus lies a sharply convoluted sulcus corresponding to the ectosylvian fissure (Figs. 2B and 3A and B). Two deep circular sulci were observed above it, which ran from the temporal cortex to the frontal aspect of the brain, namely, the suprasylvian fissure and the ectolateral fissure (Figs. 1B, D, and F, 2B, and 3A and B), which define the suprasylvian and lateral gyri, respectively. The frontal polar and orbital cortex (as defined in Morgane et al., 1980) showed a vertically running coronal sulcus close to the midline and a cruciate sulcus, delineating the anterior and posterior sigmoid gyri, running slightly obliquely toward the lateral aspect of the hemisphere (Fig. 1C) in both mysticetes. The ventral aspect of the brain was characterized by the presence of an olfactory tract abutting the olfactory lobe (lobule désert) and a series of orbital gyri distributed from the gyrus proreus medially toward the transverse gyrus of the insula laterally. The insular cortex showed a large number of parallel gyri on either side of its central sulcus (Fig. 3A and B), as well as a temporal extension as described in the bottlenose dolphin (Jacobs et al., 1984). Overall, these patterns correspond well to previous descriptions of the surface topology of the brain in mysticetes (Beauregard, 1883; Guldberg, 1885; Kükenthal and Ziehen, 1889; Ries and Langworthy, 1937; Wilson, 1938; Breathnach, 1955; Friant, 1955; Pilleri, 1964, 1966a, 1966b, 1966c; Morgane et al., 1980; Duffield et al., 1992).

The regional organization of the cerebral cortex in cetaceans remains poorly understood. Recent evidence indicates that the cetacean neocortical structure is in fact quite complex, with a degree of regional parcellation comparable to that of many terrestrial mammals (Hof et al., 2005). However, while there has been a few detailed analyses of the archicortex, paleocortex, insular, and cingulate cortex of dolphins (Jacobs et al., 1971, 1979, 1984; Morgane et al., 1982), most studies focused on rather restricted domains of cortex (Kojima, 1951; Pilleri et al., 1968; Kesarev, 1969; Kesarev and Malofeeva, 1969; Jacobs et al., 1971, 1979, 1984; Kesarev et al., 1977; Zworykin, 1977; Morgane et al., 1982, 1988; Garey et al., 1985; Garey and Leuba, 1986; Ferrer and Perera, 1988; Glezer and Morgane, 1990; Glezer et al., 1992, 1993; Hof et al., 1992; Manger et al., 1998). The structure of the cerebral cortex in mysticetes has barely been studied (Kraus and Pilleri, 1969; Jacobs et al., 1979; Morgane et al., 1982). Overall, as in odontocetes, the neocortex of *Megaptera* is thin (Figs. 3A–F and 4) and characterized by an absence of granularity, a very prominent thick layer I that is more cellular than in most terrestrial species and accounts for about one-third of the cortical thickness, the presence of occasional atypical neurons in the cell-dense layer II, and very large pyramidal neurons frequently forming clusters at the border between layers III and V (Fig. 4). The size of these pyramidal cells and their degree of clustering was helpful in defining regional boundaries. Layers III, V, and VI vary considerably in thickness, cellular density, and columnarity across regions (Fig. 4). As in odontocetes, the hippocampal formation of *Megaptera* is diminutive both in size and structural complexity (Fig. 3C and D) (Jacobs

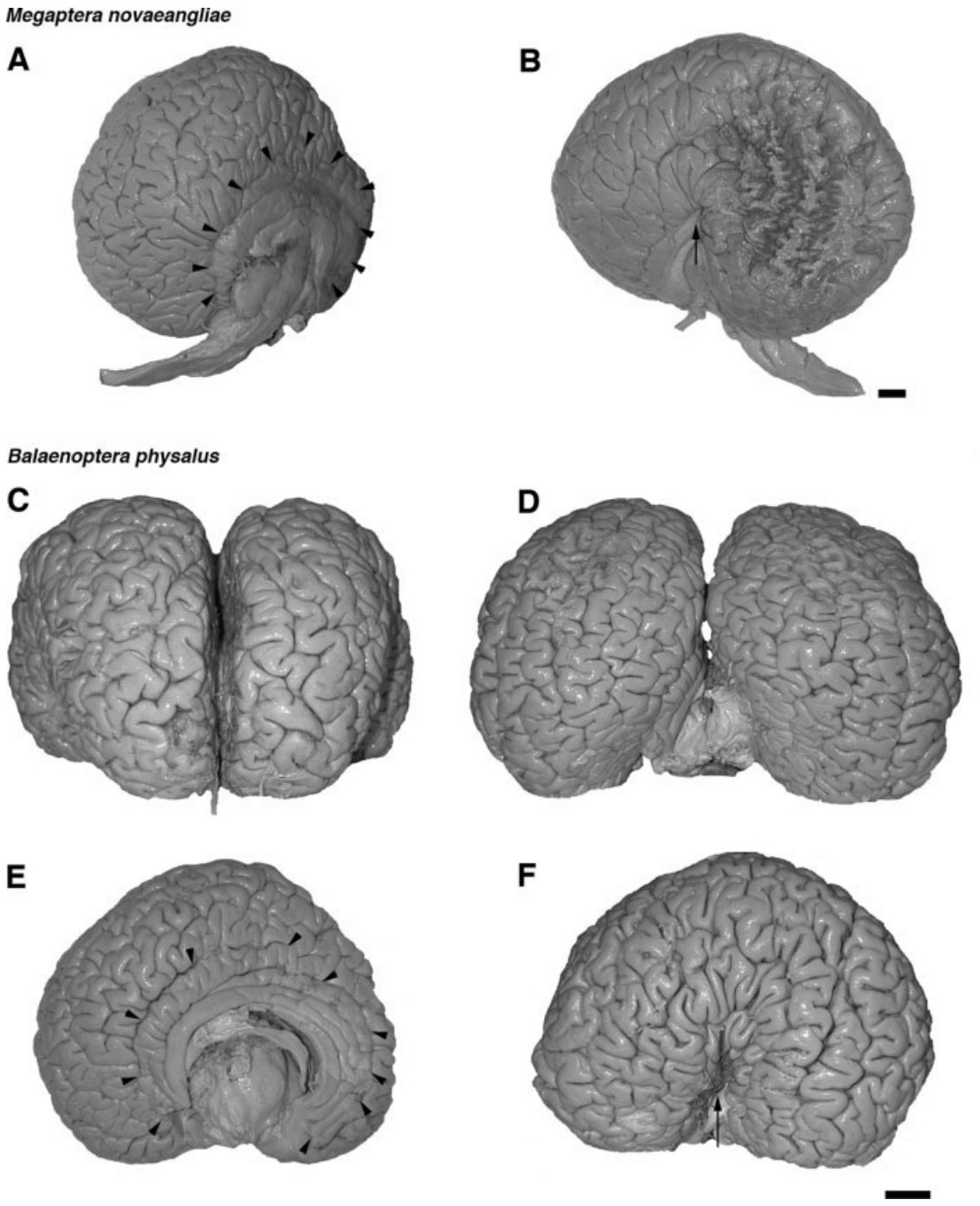

Fig. 1. Macroscopic views of the brains of the humpback whale (**A** and **B**) and the fin whale (**C**–**F**). A represents a slightly oblique view of the midline from the posterior aspect of the humpback whale brain, revealing the large occipitotemporal region. The lateral aspect of the hemisphere (B) has a damaged zone over the temporoparietal region caused during removal of the brain from the skull. The sulcal patterns are nonetheless recognizable. The hemisphere prepared for histology was intact. C shows a frontal superior view of the fin whale brain covering the possible location of the motor and somatosensory cortex. D shows the posterior view of the fin whale brain. The brainstem was cut through the quadrigeminal plate during removal. E shows the midline cortex in the same animal (anterior is to the right). Note the large limbic lobe (arrowheads; A and E). F represents the lateral view of the same hemisphere as in E (anterior is to the left). Note the verticalization of the Sylvian fissure and the concentric gyri around it (arrows on B and F and see Fig. 2B). Note the large size of these specimens and the comparable patterns of gyri and sulci distribution. Compare with Figure 2 for more details on sulcal and gyral anatomy of the humpback whale brain. Scale bar = 2 cm (A and B; *M. novaeangliae*); 3 cm (C–F; *B. physalus*).

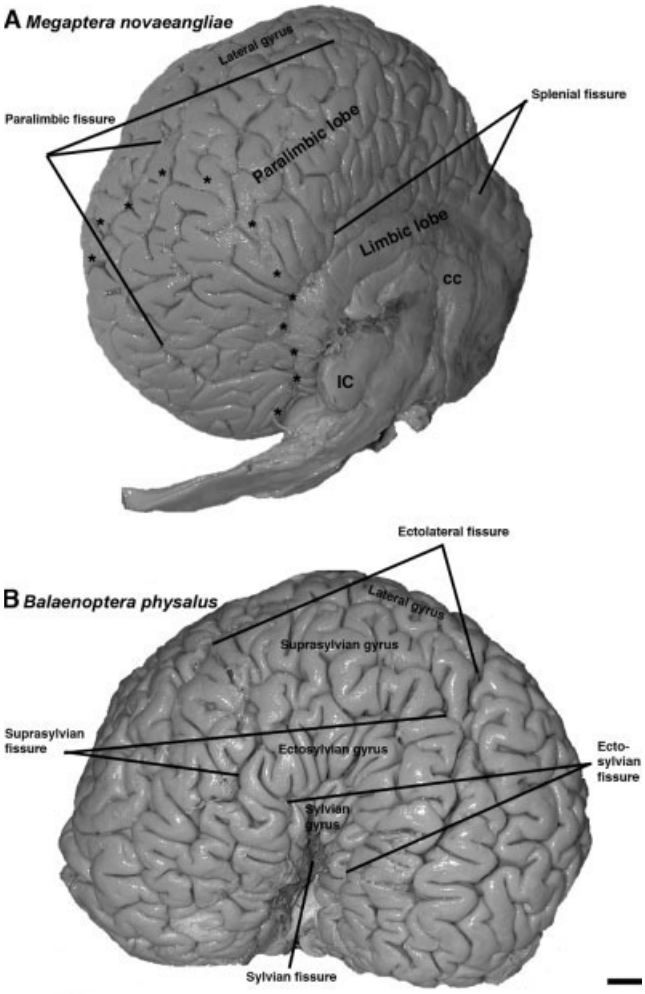

Fig. 2. Principal sulci and gyri on the brains of the humpback whale (**A**) and the fin whale (**B**). See Pilleri (1966b) and Morgane et al. (1980) for additional details. The region marked by asterisks in A represents the maximal extent of the layer II islands in the temporo-occipital region (see Fig. 17). cc, corpus callosum; IC, inferior colliculus. Scale bar = 2 cm.

et al., 1979; Morgane et al., 1982). In contrast, as described in detail below, the neocortex of the humpback whale differed from that of odontocetes in many respects, and particularly owing to the presence of an extensive posterior domain of cortex in which layer II neurons assemble in clusters fairly comparable to those seen in the entorhinal cortex of most species. Layer V of the anterior cingulate, anterior ventral insula, and frontopolar cortices also contained large elongated spindle-shaped neurons, which were also seen in the fin whale and in only two species of odontocetes with large brain and body size, the killer whale and the sperm whale, and were known thus far only from the anterior cingulate and frontoinsular cortex of hominids (Nimchinsky et al., 1995, 1999; Allman et al., 2005; Watson et al., 2006). The overall neuronal density in *Megaptera* is also much lower than in smaller odontocetes throughout the neocortex. The principal regions of cerebral cortex that are described in this study are shown in Figure 5, together with the location of most of the microphotographs shown in the plates.

Cortical Cytoarchitecture of *M. novaeangliae*

Paleocortex. The paleocortex of *Megaptera* consists of the cortical regions located in the large olfactory lobule of Broca (lobule désert) and includes the lateral olfactory gyrus, the olfactory tubercle, and piriform cortex. This anterior portion of paleocortex is limited by the frontoinsular cortex in the most anterior segment of the transverse gyrus of the insula. Medially, it abuts the posterior extent of the orbital cortex. In addition, posteriorly, the paleocortex winds around the uncus and parts of the semilunar gyrus, where the periamygdalar cortex is located. The periamygdalar cortex then merges on the gyrus ambiens with the archicortex and entorhinal cortex. Structurally, the deep layers of the paleocortex abut directly the fundus striati without clear cellular transition. The piriform area, seen on parasagittal preparations, appears as a small region, adjoining the frontoinsular cortex and recognizable from it by a thicker layer I, and less dense layers II and III containing small pyramidal cells. The cortex of the olfactory tubercle is considerably thinner, its layers much less differentiated, showing some superficial clusters of small neurons, while the deeper layer continues into the fundus striati (Fig. 6A). A number of islands of Calleja can be observed nearby and contain darkly stained, small, tightly aggregated neurons. Posteriorly, the olfactory tubercle joins the basal aspect of the septal region and the nucleus of the diagonal band, recognized by clusters of much larger, darkly stained neurons. The periamygdalar cortex is composed of relatively small granular neurons forming patches as the lamina dissecans becomes visible on the anterior aspect of the uncus (Fig. 6B). There are slightly larger and darker cells, with a better-defined superficial layer on its ventral aspect (Fig. 6C), where the pyramidal neurons' size becomes clearly larger. Its deep layers are separated from the neurons of the basal nucleus of the amygdala by a thin band of white matter on the anterior part of the uncus, but they merge directly with the amygdala ventrally.

Archicortex. In sharp contrast to the expansion of the neocortex, the hippocampus of cetaceans is noted to be relatively small and some of its components appear less well developed than in other mammals. The hippocampus in *Megaptera* is characterized by a very diminutive dentate gyrus, at places showing only a few rows of granule cells, and a very thin molecular layer (Fig. 6D). The CA3 field is rather short, with a dentate hilar component (CA4), and shows a few layers of pyramidal neurons, which are darker and larger than those in the adjacent CA1 (Fig. 6D and E). The CA2 transition is barely perceptible. The CA1 is well defined and contains two sublayers in the stratum pyramidale, a superficial layer of dense, small, hyperchromatic neurons and a thicker deep layer with larger and less densely packed cells (Fig. 6E), comparable to the situation in other mammals. The stratum oriens of both CA3 and CA1 contains many large multipolar neurons, and scattered large interneurons are seen in the stratum radiatum (Fig. 6B–E). The subiculum displays a thicker pyramidal layer with no clear sublamination pattern compared to the CA1 (Fig.

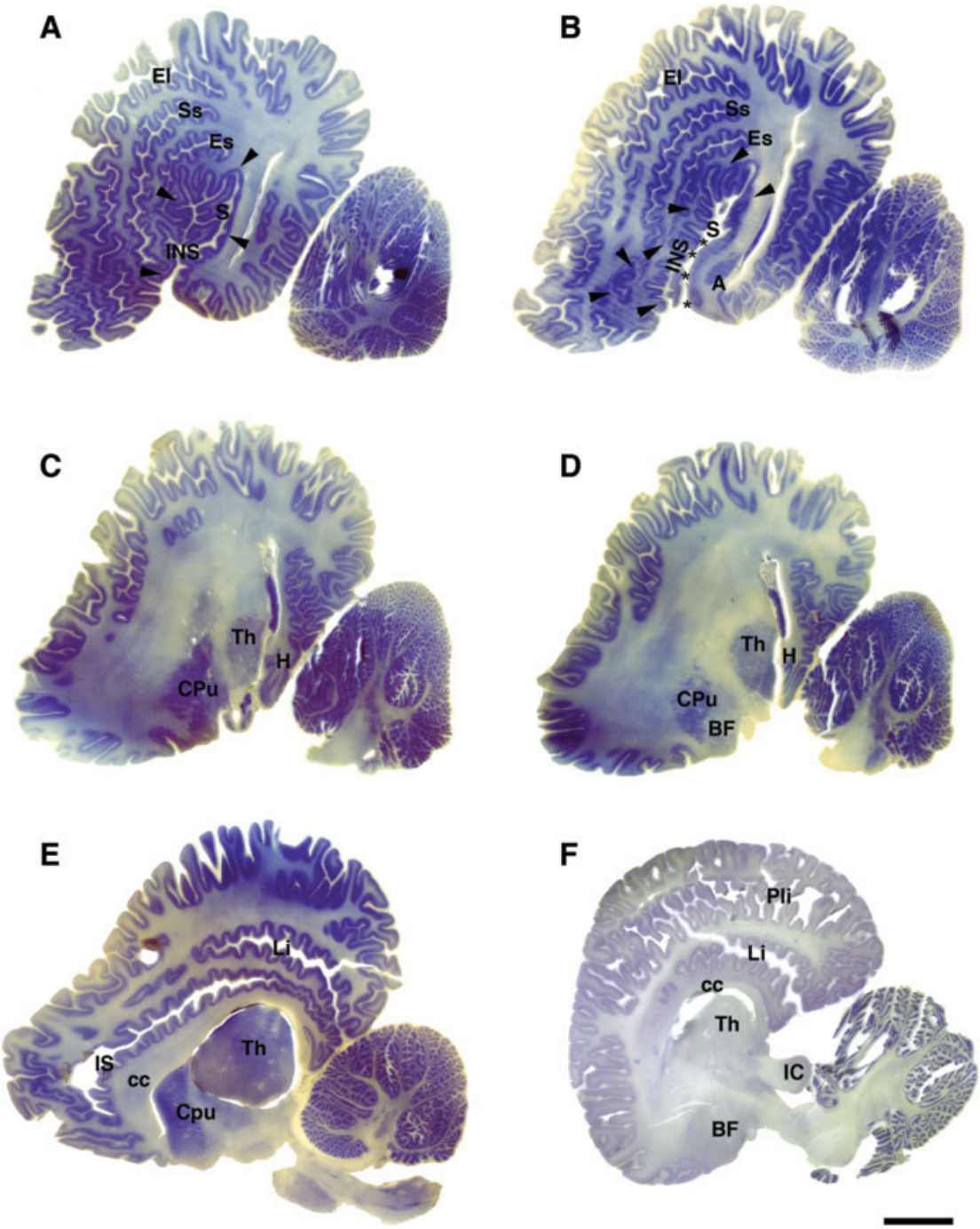

Fig. 3. Comparative series of parasagittal sections through the right hemisphere of the humpback whale (**A**–**E**) and a midsagittal level through the right hemisphere of a bottlenose dolphin (**F**). A is the most lateral and shows the Sylvian fissure and surrounding insula (arrowheads). B shows all the perisylvian sulci and gyri most clearly as well as the amygdala and periamygdalar cortex (asterisks). C and D show the basal ganglia and thalamus, as well as the hippocampal formation and entorhinal cortex. E is closer to the midline and shows the corpus callosum, the splenial fissure, and one intercalate sulcus revealing the full rostrocaudal extent of the cingulate and retrosplenial cortex. The dorsal (apical) aspect of the brain corresponds to the lateral gyrus. F shows a midsagittal section from a bottlenose dolphin to provide a comparison with the general morphology of the mysticete specimen, distribution of sulci, and convolution patterns. A, amygdala; BF, basal forebrain; cc, corpus callosum; CPu, caudate and putamen nuclei; El, ectolateral fissure; Es, ectosylvian fissure; H, hippocampal formation and entorhinal cortex; IC, inferior colliculus; INS, insula; IS, intercalate sulcus; Li, limbic (or splenial) fissure; Pli, paralimbic fissure; S, Sylvian fissure; Ss, suprasylvian fissure; Th, thalamus. Scale bar = 3 cm (A–E); 1.25 cm (F).

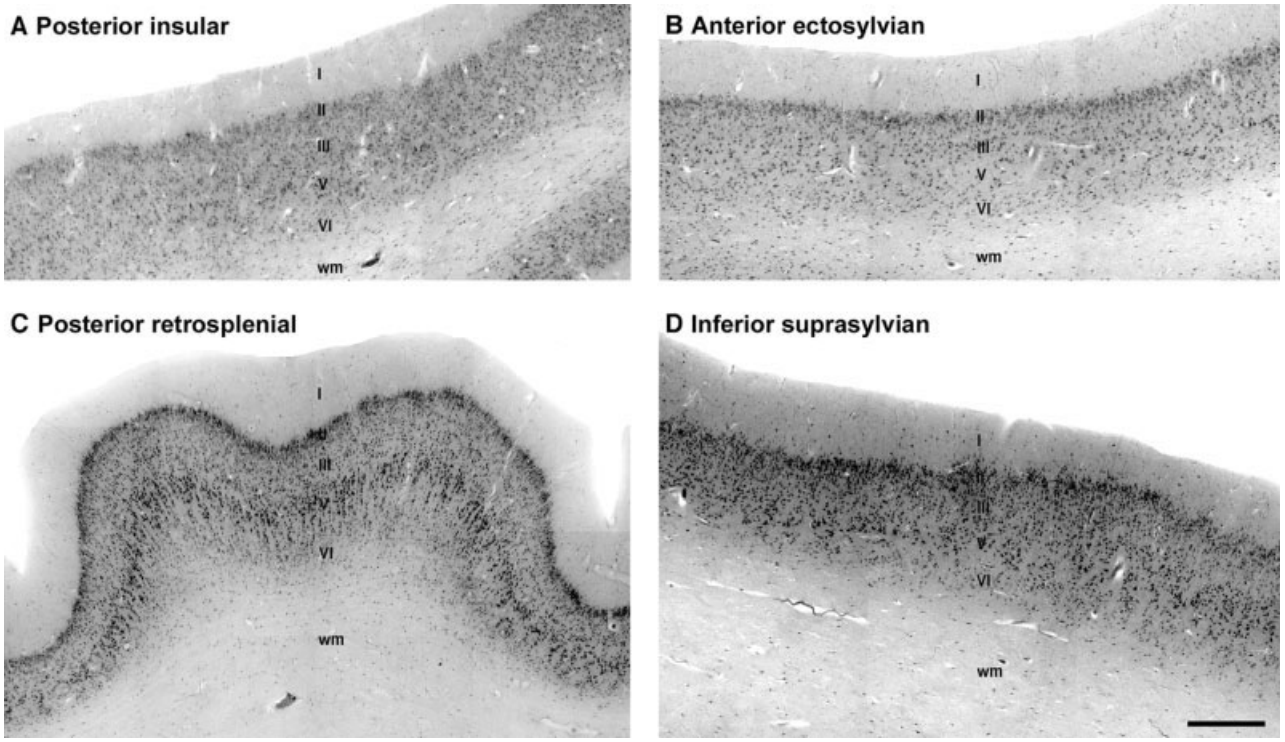

Fig. 4. Overview of neocortical layering in the humpback whale. Four different cortical locations are shown as examples of regional variability in the cellular and laminar patterns of neuronal distribution. **A:** Posterior insular cortex. **B:** Anterior ectosylvian cortex. **C:** Posterior retrosplenial cortex. **D:** Inferior suprasylvian cortex. Note the thick layer I, cell-dense layer II, the variable size and densities of pyramidal neurons in layers III and V (compare A and B to C and D), and the grouping of neurons in layers III–VI in clusters or columns depending on the region (C and D). Layers are indicated by Roman numerals; wm, white matter. Scale bar = 500 μm.

6F). Laterally, the subiculum continues into the transition cortex with neocortical regions. The presubicular field is extensive and shows a clear laminar pattern with well-defined parvocellular islands superficially, containing densely packed small pyramidal cells, a broad lamina dissecans, especially on more lateral levels in the parasagittal plane, and a deep pyramidal layer, undistinguishable from that in the subiculum (Fig. 6G). The presubiculum gets markedly thinner as it abuts the limbic component of the retrosplenial cortex, but retains this trilaminar pattern. It merges laterally with the entorhinal cortex on the parahippocampal gyrus.

Entorhinal cortex. As in most species, the entorhinal cortex of *Megaptera* can be divided into two components throughout its rostrocaudal extent: lateral and medial. It is, like the presubiculum, a large area, which appears disproportionate considering the small size of one of its major projection zone, the dentate gyrus. Dorsally and anteriorly, a large extension of the medial entorhinal cortex abuts the temporopolar cortex ventral to the temporal component of the transverse gyrus of the insula. The temporoinsular cortex at this location is much thinner, has a more regular layer II, and medium-size pyramidal neurons throughout poorly defined layers III–VI, whereas there, the entorhinal cortex appears as a thicker cortex with small, poorly defined layer II islands, a broad lamina dissecans, and highly polymorphic layers III, V, and VI (Fig. 6H). This differs from the more caudal levels of the medial entorhinal cortex, in which the layer II islands are large, more numerous, and which contains small, densely stained cells, a compact layer III with relatively large pyramidal neurons, a relatively thin but well-visible lamina dissecans, and a dense layer V–VI with regularly packed, intensely stained pyramidal cells (Fig. 6I). Laterally and caudally, on the inferior aspect of the temporal lobe, the entorhinal cortex merges with the neocortex proper. This transition is marked by a progressive spacing of the neurons in layers V and VI, a regrouping of layer II into a more continuous band of small hyperchromatic neurons, and a disappearance of the lamina dissecans (Fig. 6J).

Cingulate and retrosplenial cortex. These regions include all of the cortical domains located on the ventral bank of the splenial fissure and ventral to it on the midline of the hemisphere. In *Megaptera*, a deep external (i.e., dorsal) and a much shallower internal (ventral) intercalate sulci were present between the splenial fissure and the callosal sulcus in the anterior half of the "limbic" lobe (Figs. 1E and 2A). The retrosplenial portion had a number of relatively shallow sulci running perpendicularly to its axis. The banks of the intercalate sulci and splenial fissure were themselves convoluted (Fig. 3E). Following Morgane et al. (1982), studies in the bottlenose dolphin, the cingulate, and retrosplenial cortex can be subdivided into three general zones, the subgenual cortex in the parolfactory lobule, a supracallosal

lobule containing the pregenual, anterior, and posterior cingulate cortices, and the retrosplenial lobule containing an anterior and a posterior component. As the hemisphere of *Megaptera* was cut in the sagittal plane, a complete assessment of the midline cortex was not possible. However, adequate information about cytoarchitecture could be obtained from the cortex lying deeper in the external intercalate sulcus and splenial fissure.

Subgenual cingulate cortex. Only the most anterior and lateral aspects of the subgenual region could be examined within the depth of the intercalate sulcus. Below the thick layer I, layer II is characterized by the presence of clusters of neurons that demarcate this region from the adjacent pregenual cortex and anteriorly from the fronto-orbital cortex (Fig. 7A). Layers III and V contain a relatively homogeneous population of intermediate-size pyramidal neurons, regularly distributed with no magnocellular elements. Layer VI is thin and densely populated by small pyramidal neurons and multipolar cells (Fig. 7A).

Pregenual cingulate cortex. The pregenual region is situated directly in front of the genu of the corpus callosum. Its ventral (limbic) component has a thin, homogeneous layer II containing dense small neurons. Layers III and especially V, in contrast to the lateral subgenual cortex, contain larger but sparser pyramidal cells with an occasional magnocellular element (Fig. 7B). Layer VI displays small multipolar neurons, which tend to be organized in small clumps regularly distributed along the white matter border (Fig. 7B). The cytoarchitecture on its marginal component on the dorsal bank of the intercalate sulcus is generally comparable to its ventral component. The clustering pattern of layer VI neurons progressively disappears caudally, where the pregenual cortex merges into the anterior cingulate cortex.

Anterior and posterior cingulate cortex. The ventral aspect of the anterior cingulate cortex is characterized by a thicker layer II than that seen in the pregenual component (Fig. 7C). Layer III is thicker than in the pregenual cortex and composed of smaller pyramidal cells, while layer V contains larger neurons than layer III, which form small aggregates of 5–10 neurons. Layer VI is thicker than in the pregenual cortex and its neurons have a morphology comparable to those in the pregenual cortex but without marked clustering pattern. Dorsally on the external intercalate sulcus, the anterior cingulate cortex appears slightly thicker than ventrally and the layer V pyramidal cells form a well-visible row, at time adopting a nearly columnar stacking pattern (Fig. 7C and D). This pattern is also observed on the ventral bank of the splenial fissure, although the cortex there is thinner than around the external intercalate sulcus. The posterior cingulate cortex shows a further differentiation of layers III and V as layer V becomes thicker and the size of its pyramidal neurons increases posteriorly (Fig. 7E). The clusters of pyramidal cells in layer V also become larger in the posterior cingulate cortex, a feature particularly obvious in the external intercalate cortex and the ventral bank of the splenial fissure. Some of these patterns correspond to those reported by

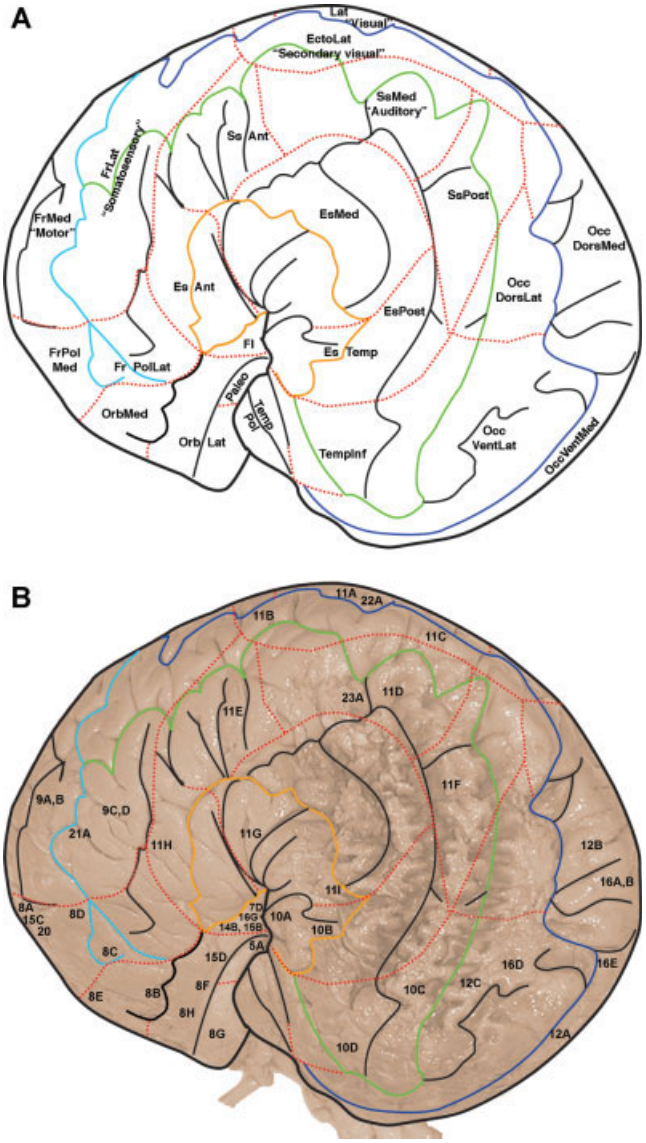

Fig. 5. **A:** Distribution of the major cytoarchitectural domains recognized in this study on the lateral aspect of the hemisphere of the brain of the humpback whale. The major sulci were traced from a picture of the lateral view of this hemisphere that is shown in anatomical position. The cruciate sulcus is shown in light blue, ectosylvian fissure in orange, the suprasylvian fissure in green, and the ectolateral fissure in dark blue. Additional sulci are shown in black. The red dotted lines depict approximate regional boundaries that are not coursing at or within sulci. No rendition was made of the midline cortex as it could not be studied on the parasagittal preparations. **B** shows the location of some of the microphotographs included in this work with the schematic outline of cortical region over a side view of the specimen's brain (same as Fig. 1B). Abbreviations of cortical fields: EctoLat, ectolateral cortex; EsAnt, anterior ectosylvian cortex; EsMed, mid-ectosylvian cortex; EsPost, posterior ectosylvian cortex; EsTemp, temporal sector of the ectosylvian cortex; FI, frontoinsular cortex; FrLat, lateral frontal cortex; FrMed, medial frontal cortex; FrPolLat, lateral frontopolar cortex; FrPolMed, medial frontopolar cortex; Lat, visual field in the lateral gyrus; OccDorsLat, dorsolateral occipital cortex; OccDorsMed, dorsomedial occipital cortex; OccVentLat, ventrolateral occipital cortex; OccVentMed, ventromedial occipital cortex; OrbLat, lateral orbital cortex; OrbMed, medial orbital cortex; Paleo, paleocortex; SsAnt, anterior suprasylvian cortex; SsMed, mid-suprasylvian cortex; SsPost, posterior suprasylvian cortex; TempInf, inferior temporal cortex; TempPol, temporopolar cortex.

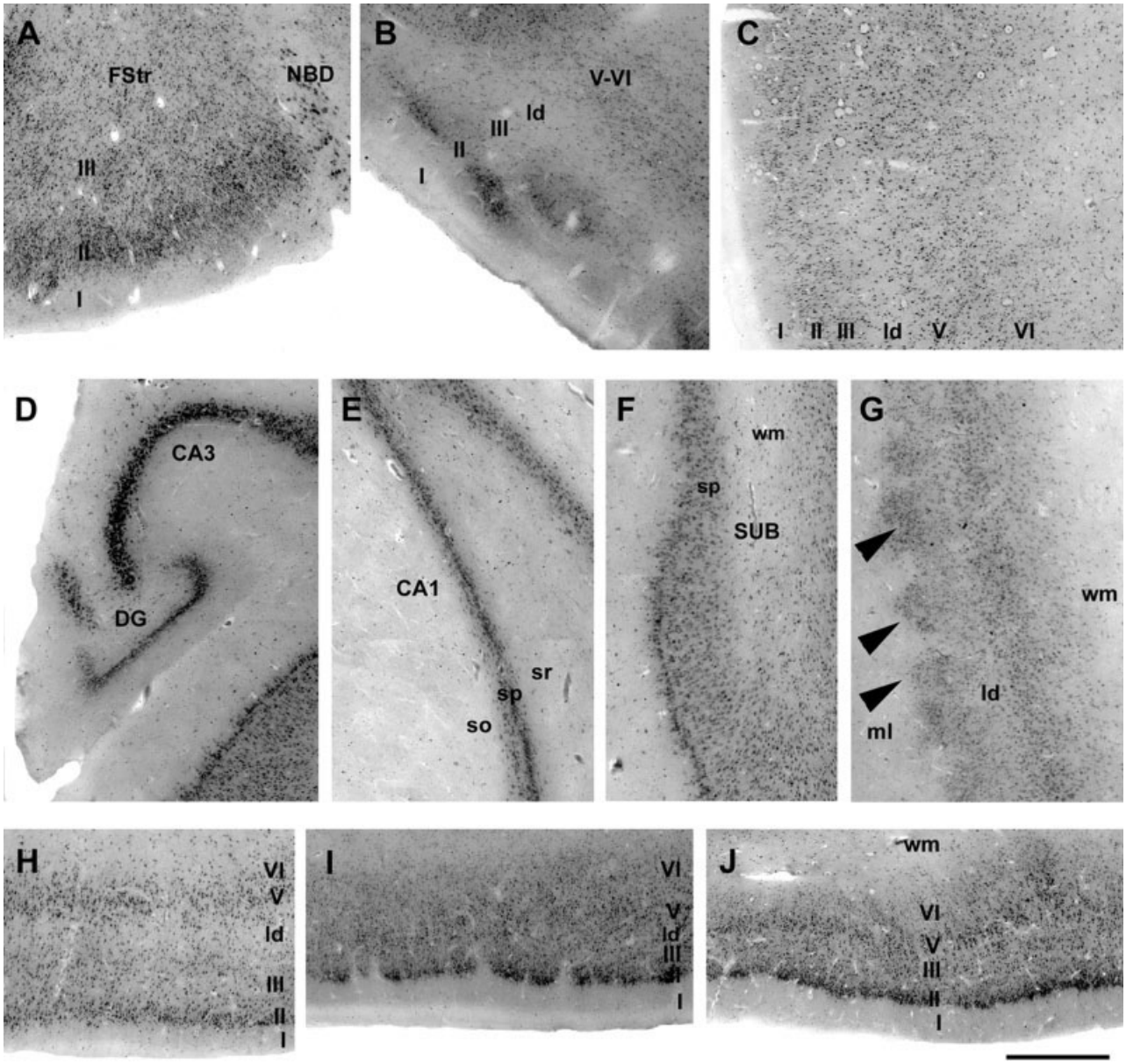

Fig. 6. Cytoarchitecture of the paleocortex, archicortex and entorhinal cortex in the humpback whale. **A:** Olfactory tubercle. **B:** Dorsal periamygdalar cortex. **C:** Ventral periamygdalar cortex. Note the three-layered organization of the olfactory tubercle (A), and the large lamina dissecans appearing in the ventral portion of the periamygdalar cortex (B and C). The cytoarchitecture of the hippocampal formation is comparable to that in other mammals, but the archicortex is overall small. Note the diminutive dentate gyrus and CA3 (**D**), the thin CA1 (**E**) and subiculum (**F**), and the thicker presubiculum (**G**) with the typical parvocellular islands (arrowheads) and a visible lamina dissecans. The entorhinal cortex (**H–J**) shows a different architecture in its anterior temporopolar portion with a clear lamina dissecans (H), compared to more posterior locations (I), where the layer II islands are best seen, and close to the temporal neocortex (J), where they disappear. Layers are indicated by Roman numerals. CA1-3, Ammon's horn fields; DG, dentate gyrus; FStr, fundus striati; ld, lamina dissecans; ml, molecular layer of the presubiculum; NDB, nucleus of the diagonal band of Broca; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum; SUB, subiculum; wm, white matter. Scale bar = 250 μm (A–G); 500 μm (H–J).

Morgane et al. (1982) in the cingulate cortex of *Megaptera*. Finally, the subgenual, pregenual, and anterior segments of the cingulate cortex all exhibit elongate, large spindle cells amid the pyramidal neurons of layer V, similar to those described in the cingulate cortex of hominids (Nimchinsky et al., 1999). A full account of this particular feature is provided in a separate section below.

Anterior and posterior retrosplenial cortex. The anterior domain of the retrosplenial cortex is marked by a thinner layer II and a lesser tendency of clustering of layer V pyramidal cells than in the anterior cingulate cortex. Layer III neurons are slender in shape than in the posterior cingulate cortex and their apparent density increases posteriorly. These changes occur as a transition

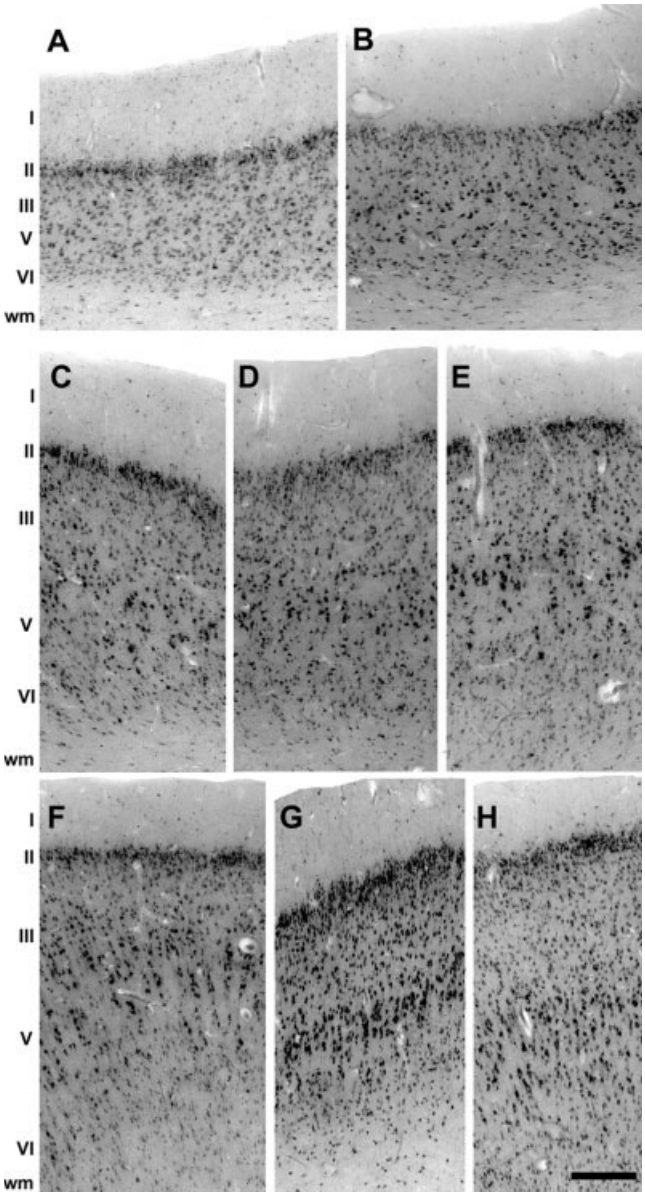

Fig. 7. Cytoarchitecture of the cingulate and retrosplenial cortex in the humpback whale. **A:** Subgenual cortex. **B:** Pregenual cortex. **C:** Ventral anterior cingulate cortex. **D:** Dorsal anterior cingulate cortex. **E:** Posterior cingulate cortex on the ventral bank of the splenial fissure. These cingulate fields can be easily recognized by their cellular organization from each other. The retrosplenial cortex (**F–H**) is markedly different from the cingulate cortex and also shows rostrocaudal (F and G) and ventrodorsal (H) gradients in its cytoarchitecture. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 200 μm.

pattern across the anterior region of the retrosplenial cortex and are more noticeable along the dorsal bank of the intercalate sulcus and the ventral bank of the splenial fissure. The posterior part of the retrosplenial cortex displays neuronal arrangements forming palisades in layers III and V, with increased cellular density compared to more anterior levels (Fig. 7F). At the most posterior levels of the retrosplenial cortex along the splenial fissure, the neuronal density increases considerably, the neurons of layer III and V become larger and more intensely stained (Fig. 7G), forming another transition zone with a cortical type comparable to that seen in the inferior temporal gyri, before abutting the most caudal extent of the entorhinal cortex, recognizable by its typical architecture. The retrosplenial cortex lining the splenial fissure dorsally is composed of very dense intermediate-size neurons (Fig. 7H), progressively merges with a region characterized again by clustering of very large pyramidal neurons in layer V, and by an island-like patterning of neurons in layer II. This region then extends posteriorly over most of the surface of the occipitotemporal cortex (Figs. 2A and 17) and is described below in more detail.

Insula. The insular cortex is distributed on the medial wall of the deep pocket formed by the Sylvian fissure (Jacobs et al., 1984). It is separated from the opercular domains of the frontal, parietal, and temporal cortices by a circular sulcus laterally and dorsally, and on its medial border the transverse gyrus of the insula abuts the anterior rhinal sulcus (Fig. 3A and B). The temporal sector on the insula merges anteriorly with the periamygdalar cortex medially and with the temporopolar cortex more laterally. The insula contains a large number of vertical gyri (17 in this *Megaptera* specimen), divided in a posterior and temporal sector and an anterior sector by the deeper central sulcus of the insula. Owing to the frontal curvature of the telencephalon in cetacean, the anterior part of the insular cortex forms a deep, ventrally oriented extension that merges with the posterior aspect of the orbital lobe (frontoinsular cortex).

The cortex of the temporal sector of the insula is overall thin and shows only a few shallow sulci. Layer II is thick but not hyperchromatic, and layers III and V appear poorly differentiable from each other, containing small pyramidal neurons. Layer VI is very thin and at places mixes with aggregates of claustral neurons (Fig. 8A). Anteriorly, the temporal sector of the insular cortex grows thicker as it reaches the periamygdalar cortex and a transitional peripaleocortical domain within the transverse gyrus of the insula characterized by layer II islands, where the lamina dissecans becomes recognizable. At more anterolateral levels, the temporopolar cortex is thicker than the insula, with identifiable layers III and V and a distinct polymorphic layer VI. The posterior sector of the insula, caudal to its central sulcus, is considerably more convoluted than the temporal sector, and its cortex is thicker. Layer II is distinguished by the presence of small clusters on neurons as previously recognized in the bottlenose dolphin (Jacobs et al., 1984; Manger et al., 1998). Layer V shows larger neurons than layer III, which associate in small groups (Fig. 8B). This clustering in layer V is more visible as one proceeds anteriorly along the insula. Layer VI is well defined, highly polymorphic, and in places intimately related to the claustrum as bridges of small fusiform neurons frequently occur between the two structures. This is observed particularly well on medial sections where the angle of cut is more favorable. The anterior sector of the insula shows deeper sulci than posterior levels. It has a relatively thin cortex, containing prominent layer V pyramidal cells in well-visible clusters (Fig. 8C). Layer II cell clusters are more marked in front of the central sulcus and are at times comparable to those seen in the

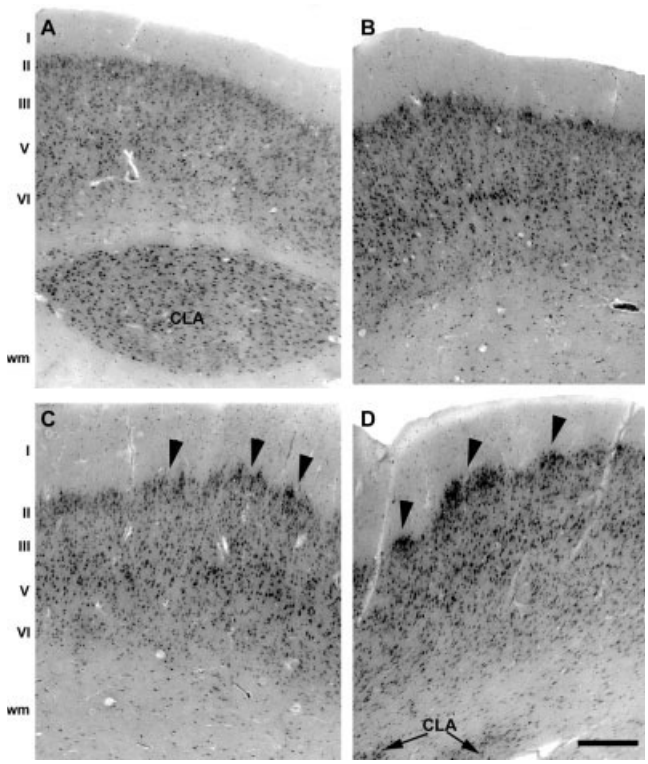

Fig. 8. Cytoarchitecture of the insular cortex in the humpback whale. **A:** Temporal sector of the insula. **B:** Posterior sector of the insula. **C:** Anterior insula. **D:** Frontoinsular cortex. The insula is characterized by a rostrally directed gradient of layer II islands differentiation (arrowheads). Note also the proximity of layer VI to claustrum (CLA) extensions around the transverse gyrus of the insula. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 200 μm.

medial entorhinal cortex (Fig. 8C; see also Fig. 17E–G). Layer III is characterized by a homogeneous population of smaller pyramidal neurons with a density comparable to that of the posterior sector. Layer VI appears thinner than in the posterior sector but presents the same relationship and features with the claustrum. The most anterior domain of insular cortex abuts the posterolateral sector of the orbital cortex and forms a frontoinsular cortex. It is a thin cortex with a sparser neuron density overall than the rest of the anterior insular segment and the frontal orbital cortex anterior to it. Layer II forms clusters of dark small neurons that are larger than in the more posterior segments of the insula (Fig. 8D). Layers III and V are not as easily differentiable as in the main portion of the anterior insula. Layer V contains rare, large, isolated pyramidal neurons. Layer VI is well developed and shows the same interactions as in the anterior sector with the claustrum (Fig. 8D). Similarly to the anterior cingulate cortex, the insula contains a large population of spindle cells in layer V. They are far more numerous anteriorly than posteriorly and are very rare in the temporal sector. They will be discussed in greater detail below.

Frontopolar and orbital cortex. This is a complex region that exhibits a double trend of cytoarchitectural organization in the polar region. Local neuronal densities indicate the presence of mediolateral and ventrodorsal gradients. Neuronal morphology and laminar makeup also differ within these gradients. The polar region itself is rather small and separated from the orbital cortex by a deep horizontally running sulcus situated at the very frontal tip of the hemisphere. The orbital cortex is itself characterized by shallower sulci and is generally thinner than the frontopolar cortex (Fig. 9A and F). Posteriorly, these regions abut the frontoinsular cortex ventrolaterally, the paleocortex ventromedially, and the subgenual and perigenual cingulate cortex dorsomedially. Dorsally on the surface of the hemisphere, the frontopolar cortex joins medially with two distinct fields of frontal cortex that may correspond to the primary motor and somatosensory regions.

Laterally, the ventral frontopolar cortex exhibits more scattered neurons in all layers compared to adjacent frontal regions. The neurons of layer II are less densely packed and the layer is thinner. The neurons in layers III and V are slender and do not aggregate as seen elsewhere. Layer VI is rather cell-sparse (Fig. 9B). Dorsally, the frontopolar cortex becomes more densely cellular with better-defined layers III, V, and VI than ventrally (Fig. 9C). Layer II shows a dense population of neurons and is thicker. Layers III and V contain intermediate-size pyramidal cells (Fig. 9C). Dorsomedially, layer V pyramidal neurons become magnocellular, forming distinct clusters, and layer II is dense (Fig. 9D). This region merges dorsally with a frontal field characterized by very large pyramids in layer V and located across the ventral extensions of the cruciate sulcus. The ventromedial aspect of the frontopolar cortex is characterized by the tendency of layer II to form cellular aggregates that become progressively more obvious posteriorly in the orbital cortex. A similar tendency is also observed more laterally toward the ventral insular cortex, where the clustering of layer II is unequivocal. Ventromedially, layers III and V consist of slightly smaller pyramidal neurons than in the dorsomedial part of the frontopolar cortex (Fig. 9E).

The orbital cortex is thin. Medially, small clusters of neurons occur in layer II and the neurons of layer III and V become smaller than in the polar region (Fig. 9F). The neurons are also sparser posteriorly toward the less differentiated pattern observed in the paleocortical regions. The cytoarchitecture of the gyrus proreus could not be assessed in these parasagittal materials. Laterally, the clustering pattern of layer II is more marked and most visible as the orbital cortex merges with the frontoinsular region (Fig. 9G). Close to the pole, the orbitofrontal cortex shows little clustering in layer II and layers III and V are cell-dense with a well-visible laminar patter, and more homogeneous than in the medial orbital cortex (Fig. 9G). A remarkable feature of the subcortical white matter immediately subjacent to the lateral extent of the frontopolar and lateral orbital cortex is the presence of a large number of claustral islands that extend as far as the dorsal aspect of the frontal pole itself (Fig. 9H), suggesting that in these species the claustrum is considerably larger than in other mammals. The islands are of variable size depending on the plane of section, and at times are directly related to cortical layer VI by cellular bridges, as seen around the insula. Finally, as described in detail below, the medial

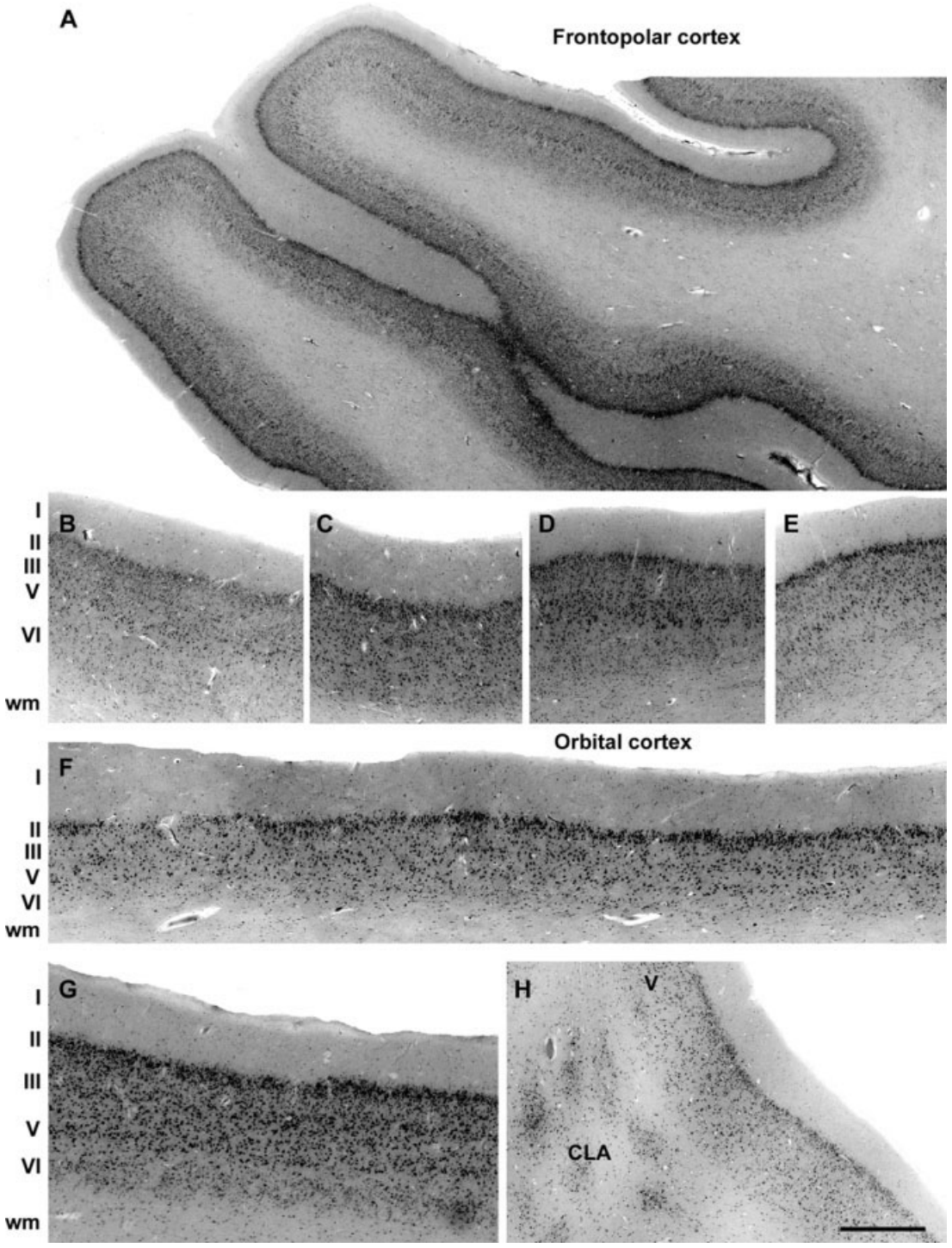

Fig. 9. Cytoarchitecture of the frontopolar (**A–E**) and orbital (**F–H**) cortex in the humpback whale. A shows a low-magnification montage of the polar gyri at the tip of the frontal lobe. This is a rather thick and highly sulcated cortex in comparison to the orbital field (F, for comparison), which is thin and shows fewer sulci. B: Lateroventral frontopolar cortex. C: Laterodorsal frontopolar cortex. D: Dorsomedial frontopolar cortex. E: Ventromedial frontopolar cortex. Note the larger layer V neurons in the dorsomedial part of the frontopolar region. The orbital cortex generally contains smaller neurons than the frontopolar cortex and shows the tendency to form islands in layer II (F and G). Layer III and V display horizontal bands of neurons (G) and layer VI is contacting claustral islands (CLA) laterally (H). Layers are indicated by Roman numerals; wm, white matter. Scale bar = 1.5 mm (A); 600 μm (B–G); 700 μm (H).

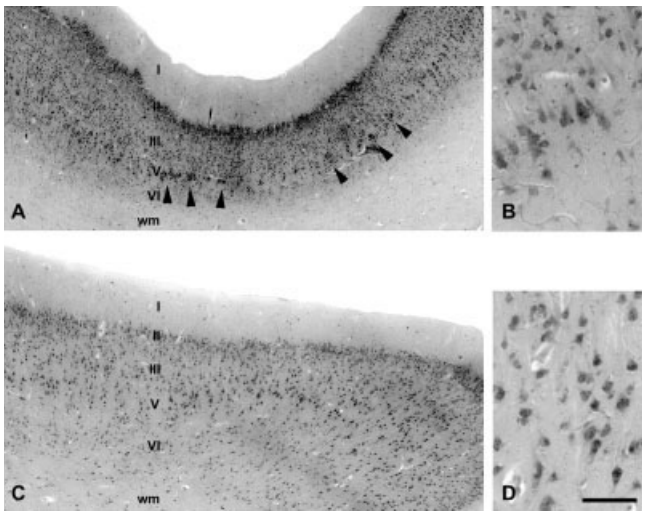

Fig. 10. Cytoarchitecture of the frontal convexity showing the putative primary motor cortex (**A** and **B**) and primary somatosensory cortex (**C** and **D**) in the humpback whale. The medial frontal convexity contains a magno/gigantocellular cortex with distinct layer V clusters (A; arrowheads), whereas laterally the frontal cortex displays much smaller neurons in layer V (C) and compare cellular details in B and D. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 600 μm (A and C); 100 μm (B and D).

frontopolar cortex, and to some extent the medial orbital cortex, exhibits a population of spindle cells in layer V, which are comparable in morphology to those seen in the anterior cingulate and insular cortices.

Cortical architecture of frontal convexity. A vast extent of the medial part of the frontal convexity is characterized by a magnocellular/gigantocellular type of cortex. Layer II is thick and very dense. Layer III contains intermediate-size neurons, in high densities, and exhibiting a regular distribution in the layer. Layer V stands out due to the presence of very large pyramidal neurons, forming clusters of at times 15 or more neurons (Fig. 10A). Layer VI is thick with large neurons showing a vertical arrangement (Fig. 10A). This organization is clearly seen from the midline along the coronal sulcus to the frontal extension of the ectolateral fissure laterally. In the dorsal direction, it extends from the ventral-most reaches of the cruciate sulcus just above the frontal pole itself, to approximately the apex of the hemisphere on the lateral gyrus, where the cortex becomes thinner and the very large pyramidal cells of layer V progressively disappear. The largest layer V neurons are observed in the lateral gyrus (Fig. 10B), in the more medial part of this magnocellular field. It is interesting to note that this region of cortex is quite homogeneous and comparable in size to the frontal distribution of magnocellular neurons described by Kojima (1951) in the sperm whale. Also, the part of this region that contains the largest cellular elements has a location reminiscent of the cortical zone recognized as a primary motor area in the bottlenose dolphin (Lende and Akdikmen, 1968).

Lateral to this magnocellular region, the cortex in the depth of the ectolateral fissure and lateral to it in the suprasylvian gyrus as well as in the part of the posterior sigmoid gyrus shows much fewer large pyramidal cells (Fig. 10C). Rather, layers III and V are less well defined, both containing intermediate-size neurons (Fig. 10D), layer V exhibiting a few isolated large pyramidal cells. Layer VI is thick but lacks the columnar pattern seen in the magnocellular cortex, and layer II is less densely packed (Fig. 10C). This large region is fairly homogeneous in cytoarchitecture and extends laterally to the cortex within the dorsal bank of the ectosylvian gyrus anteriorly, and dorsally, it ends approximately at the apex of the frontal convexity, similar to the magnocellular field. At its lateral and posterior boundaries, layer V becomes again more conspicuous with a certain degree of clustering of large neurons, differentiating the frontal lateral cortex from a temporoparietal type of cortex. This parvocellular frontal field (by comparison to the magnocellular cortex described above) may correspond based on location to the possibly somatosensory region described physiologically by Lende and Welker (1972) in the bottlenose dolphin.

Temporo-parieto-occipital cortex. This region is composed of a complex expanse of cortex that comprises the opercular and supralimbic (sensu Morgane et al., 1980) perisylvian areas on the lateral side of the hemisphere posterior to the frontal region representing the possible motor and somatosensory cortices, up to the lateral gyrus at the apex of the brain (Fig. 10). In this large cortical domain, the cortex is distributed roughly along the posterior two-thirds of the ectosylvian, suprasylvian, and ectolateral fissures, including their extensions over the inferior (occipital) surface of the temporal lobe. Whereas no clear subdivision between the temporal and parietal cortex can be made owing to the concentric disposition of the gyri and sulci around the Sylvian fissure and to the fact that cytoarchitectonic boundaries would likely straddle any arbitrary division, we will nevertheless consider as temporal the part of the cortex below a horizontal line positioned at the tip of the Sylvian fissure, the brain being viewed in its anatomical position in the skull, and as parietal the cortex above this line. General patterns of the cytoarchitecture of these regions are given below.

Temporal region. Immediately adjacent to the insula, the Sylvian cortex is thin and characterized by a rather loose layer II, indistinct layers III and V showing intermediate- and small-size pyramidal neurons with no large neurons in layer V, and a thin, cell-sparse layer VI (Fig. 11A). This pattern changes when the ectosylvian fissure is reached. There, the cortex is thicker and exhibits a denser layer II and a thicker, well-defined layer VI (Fig. 11B). Layers III and V remain fairly homogeneous with intermediate-size neurons and no evidence of clustering of neurons in layer V as in the Sylvian cortex (Fig. 11B), except in its most dorsal part, where the cortex becomes markedly thinner and shows distinct clusters of small layer V pyramidal cells as it joins the parietal region. The cortex along the most lateral extensions of the suprasylvian fissure (Fig. 11C), posterior and dorsal to the ectosylvian cortex, shows a thin and dense layer II, which in the most posterolateral aspect of the temporal convexity meets with the layer II clusters of the inferior temporo-occipital cortex, a cellular density in layer III comparable to that in the ectosylvian

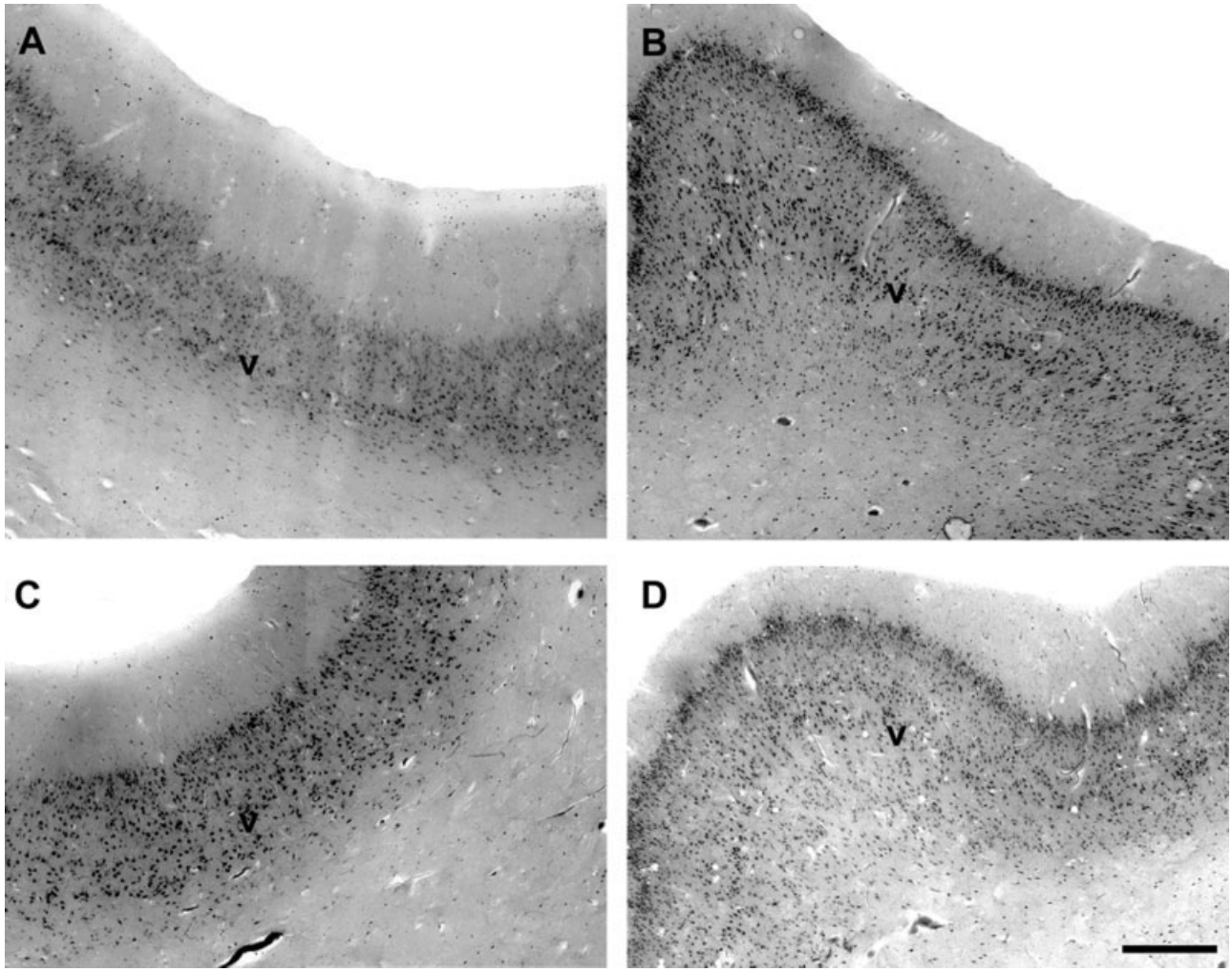

Fig. 11. Cytoarchicture of the temporal lobe in the humpback whale. **A:** Sylvian cortex. **B:** Ventral ectosylvian cortex. **C:** Suprasylvian cortex along the posterior bank of the temporal extension of the suprasylvian fissure. **D:** Lateral inferior temporal cortex. Note the variability in cell size in layer V and local differences in neuronal densities that permit the definition of these rather broad domains of cortex. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 500 μm.

cortex, but a better-defined and thicker layer V with the presence of a few isolated magnocellular cells, whereas layer VI is thin and cell-sparse (Fig. 11C). Dorsally toward the parietal cortex, the temporal cortex becomes magnocellular with the appearance of larger pyramidal neurons in layer V arranged in small clusters and a progressive columnar organization in layers III and VI. Anteriorly on its inferior surface, the temporal cortex abuts the entorhinal cortex. The lateral third of the inferior temporal cortex is a rather homogeneous region characterized by a relatively thick cortex with a prominent layer II and cell-sparse layers III to VI devoid of large pyramidal neurons (Fig. 11D). Medially, the inferior temporal cortex is thicker and has a higher cellular density than laterally, but remains parvocellular until it meets the occipital zone with layer II clustering, at which point larger pyramidal neurons in layers III and V become apparent.

Parietal region. At the apex of the hemisphere in the lateral gyrus, a large domain of cortex is characterized by a relatively low density of neurons compared to the neocortex of the frontal and temporal lobes, a thin layer II and cell-sparse layer III containing small pyramidal neurons and small clusters of intermediate-size pyramidal cells in layer V with occasional large elements. Layer VI in this region is thick, with cells arranged in small modules with well-visible gaps among them (Fig. 12A). This field extends over the ectolateral fissure laterally, into the suprasylvian cortex. Based on its localization and available literature in cetaceans (Sokolov et al., 1972; Ladygina et al., 1978; Supin et al., 1978; Garey and Revishchin, 1989; Revishchin and Garey, 1990), it may correspond to the primary visual cortex. Anteriorly, to this region the cortex is thin, with a dense layer II, small cells in layer III, a cell-sparse layer VI with less well-defined modules, and few small

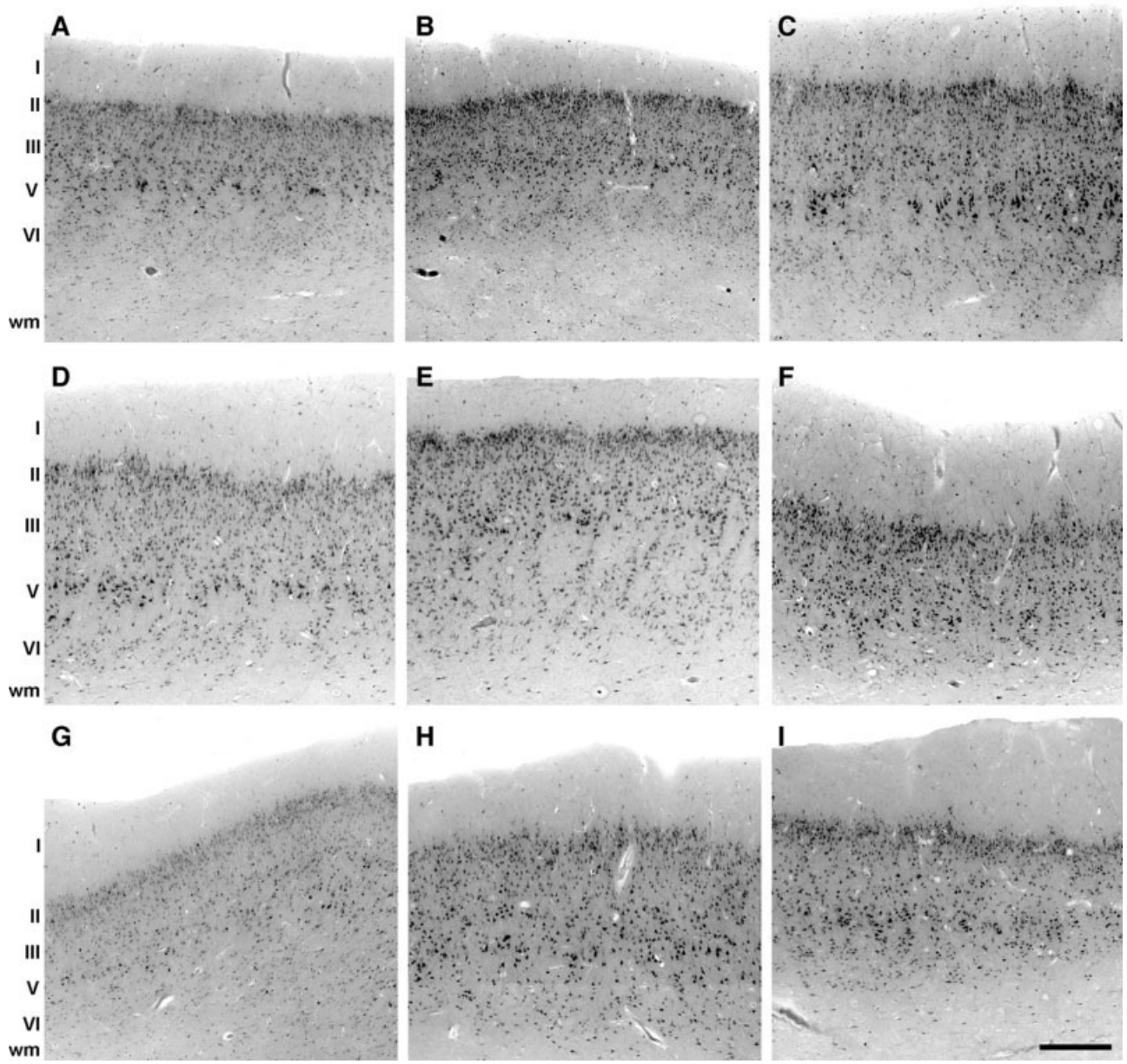

Fig. 12. Cytoarchitecture of the parietal cortex in the humpback whale. This large region can be divided overall in lateral (**A–C**), suprasylvian/ectolateral (**D–F**), and sylvian/ectosylvian (**G–I**) domains from the midline to the Sylvian fissure, and each of these "tiers" shows an anterior (B, E, and H), middle (A, D, and G), and posterior (C, F, and I) component. The middle cortical domains on the lateral gyrus (A) and suprasylvian/ectosylvian cortex (D) show a characteristic layers V–VI pattern of neuronal columnarity and neuropil patches. These fields may correspond to the primary visual and primary auditory cortex, respectively. The cortices anterior and posterior to these two regions show a less marked pattern in layers V and VI and are likely to be secondary sensory fields arranged around the primary region (B, C and E, F). Such pattern is not seen in the opercular cortex near the Sylvian fissure (G and H), but occurs to some degree in the posterior ectosylvian cortex near the temporal cortex (I). Layers are indicated by Roman numerals; wm, white matter. Scale bar = 300 μm.

clusters of relatively small pyramidal neurons in layer V (Fig. 12B), which contrasts sharply with the magnocellular field that it abuts in the frontal lobe. Posterior to the putative primary visual area, the lateral and ectolateral cortex show a loose layer II but larger neurons in layers III and V, with clear clusters in layer V, and a thick heterogeneous layer VI (Fig. 12C). This type of cortex is seen to continue around the medial field in the suprasylvian cortex, into the suprasylvian fissure, possible representing visual association areas. This belt-like structure is indeed comparable to the organization of perivisual fields as seen in the carnivore brain, which have a similar perisylvian distribution of gyri, such as the cat (Otsuka and Hassler, 1962; Montero, 1981; Sherk, 1986;

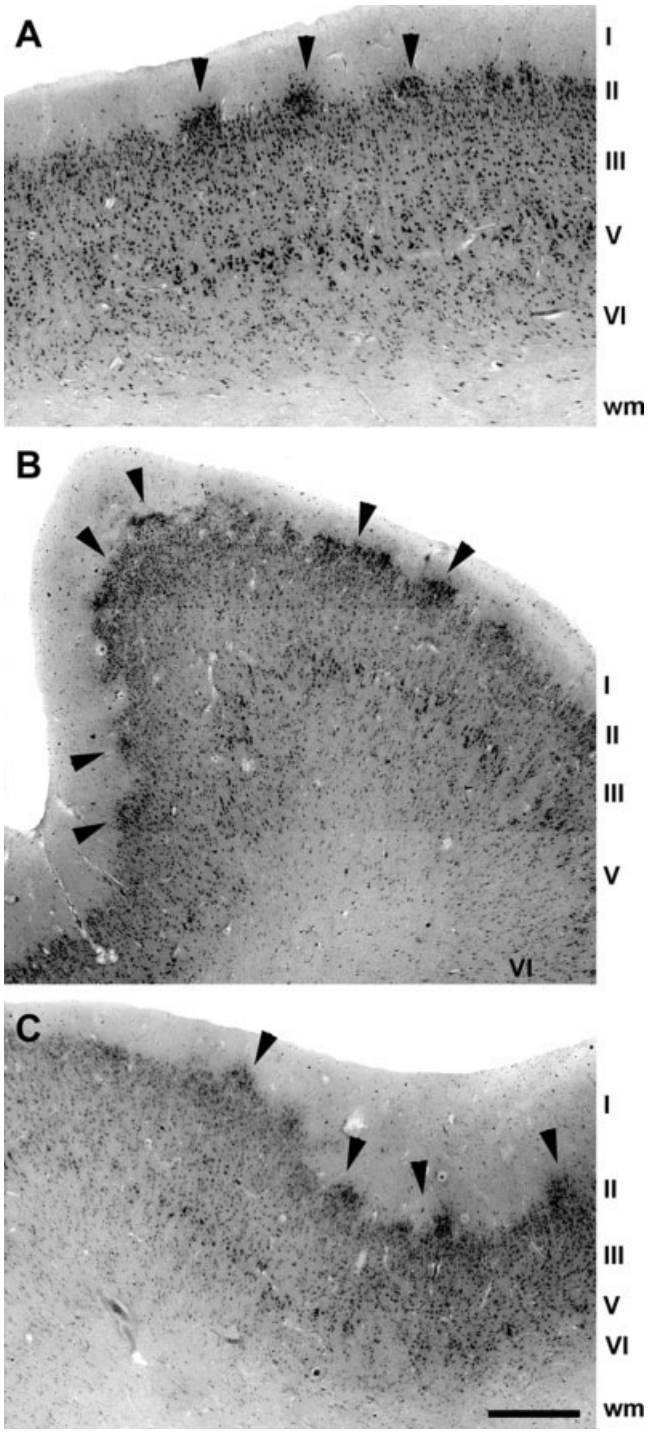

Fig. 13. Cytoarchitecture of the occipital lobe in the humpback whale. A large domain of the occipital convexity is characterized by the presence of well-visible cellular modules in layer II (arrowheads) that have a variable size and density (see also Fig. 17). Ventromedially (**A**), the occipital cortex contains larger clusters of neurons in layer V than dorsomedially (**B**) and ventrolaterally (**C**). Layer V neurons tend to aggregate under the layer II islands in these latter regions. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 400 μm (A); 500 μm (B and C).

Felleman and Van Essen, 1991; Payne, 1993; Scannel et al., 1995; Van der Gucht et al., 2001; Lee and Winer, 2005).

Laterally in the suprasylvian cortex and into the ectosylvian cortex, a different pattern of cortical organization emerges, characterized by low cellularity across layers V and VI, and by a more apparent stacking of neurons in columns or cortical modules than elsewhere in the cortex. Medially, this cell-sparse cortex shows a thick layer I, an homogeneous thin layer II, columns of intermediate-size pyramidal cells in layers III and V, and a thick layer VI, also displaying a columnar arrangement of neurons (Fig. 12D). Anteriorly, the cortex becomes slightly more cell-dense than in this medial domain and exhibits small clusters in layer V with larger pyramidal neurons. Layer VI is quite sparse and the cortex exhibits a similar columnarity as in the middle tier (Fig. 12E). Posteriorly, the cortex shows a very thick layer I, a loose layer II, a similar layer III as in the middle tier, but the presence of magnocellular elements in layer V with a more marked clustering of pyramidal cells and a less conspicuous pattern of neuronal stacking than in the middle region (Fig. 12F). These regions are in a position to represent the primary auditory cortex and around it some auditory association regions (Sokolov et al., 1972; Ladygina et al., 1978; Supin et al., 1978; Krasnoshchekova and Figurina, 1980; Voronov et al., 1985). It is interesting to note that both the putative auditory and visual cortices share a remarkable alternating pattern of columnar groups of neurons and large patches of neuropil that may represent incoming thalamic afferents to these auditory and visual regions.

The opercular part of the parietal cortex in the ventral ectosylvian and directly perisylvian cortex differs considerably from the adjacent putative auditory cortex. It is much denser, with a thin, densely packed layer II and a sparse layer VI. Layers III and V contain intermediate- and small-size pyramidal neurons and are difficult to differentiate from each other (Fig. 12G). Anteriorly toward the sensory areas of the frontal lobe, layer II becomes thicker. Layers III and V remain densely packed and larger neurons are seen in layer V with a certain degree of clustering, while layer VI exhibits low densities of neurons (Fig. 12H), differentiating this cortex from that in the lateral aspect of the frontal region. Posteriorly, where the ectolateral cortex continues into the temporal lobe, these regions exhibit a rather thin cortex, with a thin, dense layer II, a sparse layer III, and the presence of well-defined clusters of small pyramidal cells in layers V and VI (Fig. 12I). This pattern then disappears in the temporal lobe.

Occipital region. Based on our definition of the temporal and parietal cortex, the occipital domain then represents the posterior polar cortex and part of the inferior (temporal) aspect of the hemisphere that covers the anterior surface of the cerebellum. It also corresponds generally to the region characterized by clustering of layer II neurons (Figs. 2A and 17). Overall, comparable gradients of cellular density and cell size are present over this region as in the inferior temporal cortex described above. Laterally, the cortex is sparser and parvocellular, whereas medially, it is denser and larger neurons appear

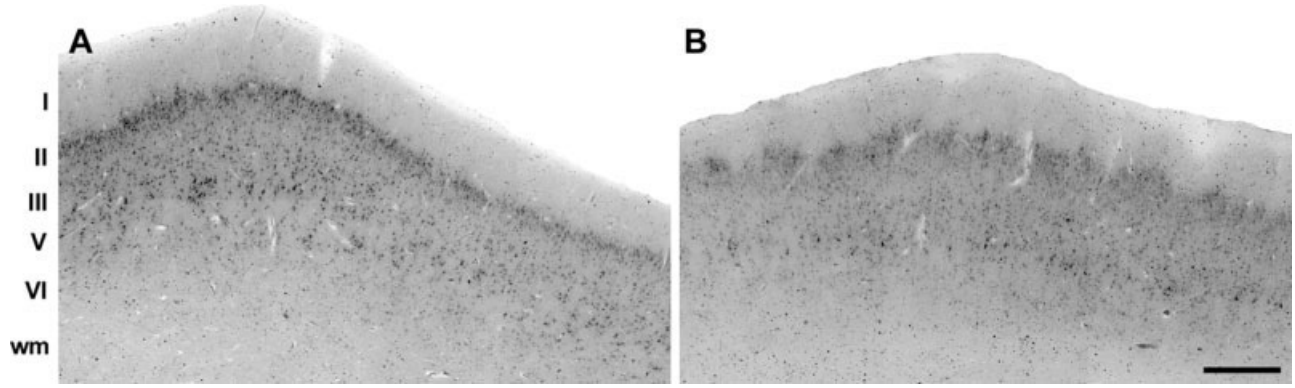

Fig. 14. Cytoarchitecture of the paralimbic cortex in the humpback whale. This is a thin and rather cell-sparse cortex with few layer V neuron clusters in its anterior portion (**A**) compared to its posterior segment (**B**), where layers V and VI display clear aggregates of pyramidal cells. Layer II posteriorly also shows the modularity seen over the occipital convexity (B). Layers are indicated by Roman numerals; wm, white matter. Scale bar = 450 μm.

in layers III and V, forming large clusters in layer V (Fig. 13A). In the dorsal direction, the cortex also shows progressively larger pyramidal neurons. The clusters in layer II are smaller in the lateral and ventral aspect of the region (Fig. 13A), whereas they are much larger dorsally and medially, where the layer V pyramidal cells are the largest and are organized in aggregates generally aligned under the layer II clusters (Fig. 13B). Ventrolaterally, layers III and V contain intermediate-size pyramidal neurons (Fig. 13C). The posterior polar aspect of the suprasylvian and ectolateral cortex is deeply convoluted. It exhibits the largest layer II clusters, very large elongated layer V pyramidal cells, and a certain degree of columnar arrangement of layers III and VI as well, not seen in ventral and lateral part of the temporo-occipital region (Figs. 13B and 17). Dorsally toward the parietal cortex, the clustering of layer II shows progressively smaller islands and eventually disappears.

Paralimbic lobe. Owing to the parasagittal plane of section of the available materials, only the cortex within the depth of the posterior half of the paralimbic fissure and on the posterior surface of the hemisphere could be reliably analyzed in this specimen. The paralimbic cortex toward the middle third of the paralimbic fissure is thin (Fig. 14A), with a relatively sparse layer II, a moderately dense layer III made of rather small pyramidal cell, and intermediate-size pyramidal neurons in layer V, with occasional larger cells, not forming as many clusters as elsewhere. Layer VI is thin and cell-sparse (Fig. 14A). Posteriorly, the cortex on the surface of the paralimbic lobe exhibits the layer II clustering seen in other part of the occipital region (Fig. 14B). At these levels, corresponding to the posterior pole of the region homologous to the lingual lobule of odontocetes (Morgane et al., 1980), up to the occipital surface of the lateral gyrus, the cortex is thicker than in the paralimbic fissure, with larger neurons in layers III and V. Pyramidal cells in layer V aggregate into small clusters with larger neurons, and layer VI is thicker and better defined than at more anterior levels (Fig. 14B).

Cortical Specializations in *M. novaeangliae*

Spindle cells (von Economo neurons). Spindle cells or Von Economo neurons were originally described in humans (Von Economo, 1926; Nimchinsky et al., 1995, 1999) and are found only in the cortex anterior cingulate gyrus (Nimchinsky et al., 1995) and in the frontoinsular cortex (Allman et al., 2005; Watson et al., 2006). They were subsequently described in great apes to the exclusion of all other primate and available mammalian species (Nimchinsky et al., 1999). However, the neocortex of mysticetes was not explored at that time. These neurons are characterized by a very elongate, tapering, large-size perikaryon mostly symmetric about its vertical and horizontal axes, and extensive apical and basal dendrites. Some dendrites may be truncated shortly after the perikaryon, ending in a brush-like pattern (Nimchinsky et al., 1995, 1999). They are usually lightly stained on Nissl preparations compared to the surrounding pyramidal neurons (Nimchinsky et al., 1995, 1999; Watson et al., 2005). These studies also demonstrated that the spindle cells represent a class of projection neurons that send an axon to the subcortical white matter and likely contribute to the connectivity of the prefrontal cortex and select subcortical centers (Nimchinsky et al., 1995, 1999; Allman et al., 2005; Watson et al., 2006).

Whereas spindle cells were not observed in a couple of odontocete species that had been looked at in our previous study (Nimchinsky et al., 1999), it was our surprise to observe a large number of these neurons in the neocortex of *Megaptera*. Importantly, spindle cells in *Megaptera* are distributed in the cortical regions homologous to those where they were first discovered in great apes and humans, namely, the entire anterior cingulate cortex and in the frontoinsular cortex (Fig. 15A and B). In comparison to hominids, spindle cells occupy a larger portion of these regions in *Megaptera*. In humans and great apes, they are restricted to layer Vb in Brodmann's cingulate subareas 24a, 24b, and 24c, in subcallosal area 25, being most abundant in the cortex of the medial wall of the cingulate gyrus (subarea 24b) (Nimchinsky et al., 1999), and to the frontoinsular cortex, a diagonally oriented band of cortex (as seen in the coronal plane) at the junction between the posterior orbito-

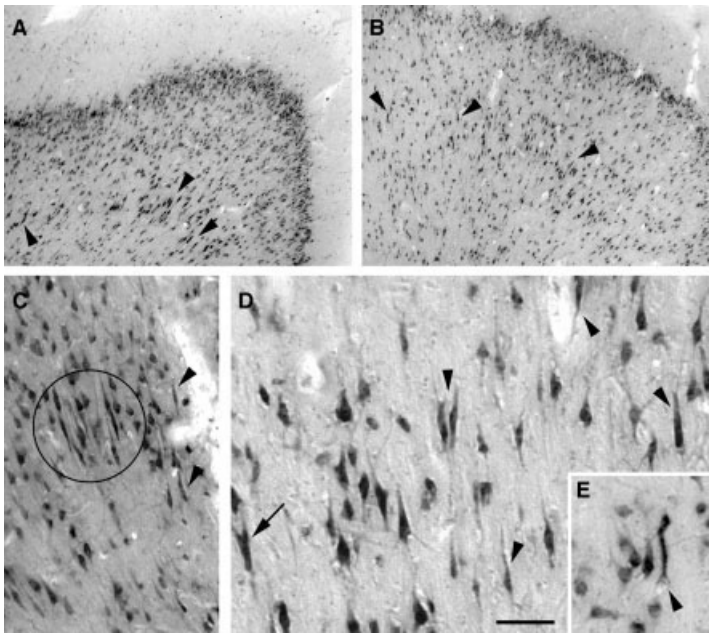

Fig. 15. Spindle cells in the humpback whale neocortex. A large number of spindle cells (arrowheads) are found in the anterior cingulate (**A**) and insular cortices (**B**). Note their elongate morphology with clearly visible apical and basal dendrites (**C** and **D**; arrowheads), which may divide in some examples (D; arrow), and grouping in small clusters (C; circle). A few also have blunted dendrites with the appearance of brush-like endings on the basal dendrite (**E**; arrowhead). The group of spindle cells in D is also visible in the center of Figure 16B, and the spindle cell shown in E is marked by the left arrowhead in A. Scale bar = 300 μm (A and B); 100 μm (C); 60 μm (D and E).

frontal plane and the ventral anterior agranular insula (Allman et al., 2005). From a morphological point of view, the mysticete spindle cells tend to be slightly smaller than, and not as thin as, those in humans, looking more like those described in orangutans and gorillas (Fig. 15C and D; see Fig. 19E for comparison with human spindle cells) (Nimchinsky et al., 1999). They show elongate apical and basal dendrites and are slightly more darkly labeled by the Nissl stain than is the case in hominids (Fig. 15C and D). Some display atypical apical dendrites that divide into two branches, and blunting of one dendrite also occurs as seen in hominids (Fig. 15D and E). They also tend to be primarily localized in layer V, with a few in layer III, and comparable to their distribution in humans and pygmy chimpanzees, they frequently associate in small clusters in the humpback whale (Fig. 15C). It was striking that in *Megaptera*, spindle cells are preferentially located at the crown of gyri and less so in the cortex along the banks of sulci. This may be related to the extensive convolution of the cetacean brain. This distribution pattern cannot be the result of misinterpreted pyramidal neurons sectioned at an odd angle for a number of reasons: these cells show the typical dendritic extensions of spindle cells known from hominids; typical pyramidal neurons occur at the crown of gyri next to spindle cells; and, reciprocally, typical spindle neurons are observed together with pyramidal neurons in the cortex lining sulci (Figs. 15A and B and 16).

In *Megaptera*, spindle cells are seen in the subgenual and pregenual and the entire supracallosal portion of the anterior cingulate cortex. They are especially numerous in the pregenual domain along the depth of the cortex in the intercalate limbic sulcus (Fig. 16A). They are more numerous in the "limbic" gyrus, closer to the corpus callosum, than in the marginal gyrus immediately bordering the splenial fissure. A few spindle cells are present in the cortex of the ventral bank of the splenial fissure and they disappear completely in its dorsal bank in the paralimbic cortex. In addition to this ventrodorsal gradient in spindle cell density, their distribution exhibits a rostrocaudal gradient in the anterior cingulate cortex, as seen in hominids (Nimchinsky et al., 1995, 1999), with apparent numbers decreasing steeply toward the posterior segment of this region (the cingulate "motor" regions in primates, in which spindle cells are not observed). It should be cautioned that our celloidin preparations in the sagittal plane did not allow us to survey the cortex of the midline and as such our observations are limited to the cortex along the intercalate and splenial sulci. However, opportunistic analysis of the cingulate cortex in a fin whale, in which the midline could be sampled, confirms the presence of spindle cells in the medial wall of mysticetes. In the insula, spindle cells are most numerous in the ventral anterior segment of the insula and in the frontoinsular cortex (Fig. 16B). They show progressively decreasing densities in the middle segment of the insular cortex in the vertical gyri toward the central sulcus of the insula. They are rarely observed in the posterior insula and the temporal extension of the insula. As such, they display a comparable rostrocaudal distribution in the insula as they do in the anterior cingulate cortex. Similarly, they are also more frequent ventrally than dorsally near the circular sulcus.

In addition, spindle cells are also present in the polar portion of the frontal cortex in *Megaptera*, a location where they were not observed in hominids, issues of

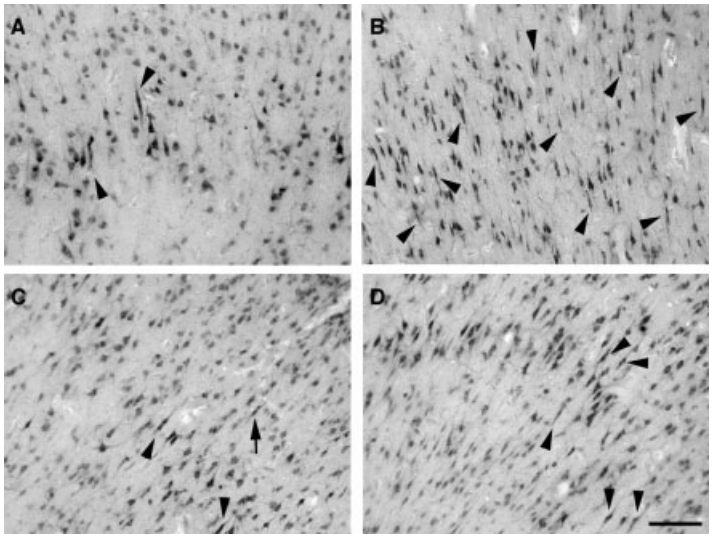

Fig. 16. Distribution and local densities of spindle cells in the humpback whale neocortex. Spindle cells are most numerous in the pregenual cingulate cortex (**A**) and frontoinsular cortex (**B**). They are also encountered in lower densities at the tip of the frontopolar region (**C**) and along the orbital gyri (**D**). Scale bar = 100 μm.

homology of cortical organization aside. In this region, spindle cells occur in a restricted domain of cortex around the tip of the frontal cortex, predominating medially with respect to the hemisphere (Fig. 16C), and along a couple of deep longitudinal sulci penetrating the orbital part of the hemisphere (Fig. 16D). The spindle cells are progressively less numerous laterally, and they are not seen in the magnocellular cortex of the frontal lobe dorsally to the medial polar region. They are more numerous around the frontal pole than along the orbital sulci and we could not ascertain whether this population of frontal spindle cells is continuous with that located in the frontoinsular cortex. However, it is possible that in mysticetes, their overall distribution defines a cortical "core" extending from the frontoinsular cortex rostrally to the tip of the orbitofrontal pole and dorsally to the posterior insular and cingulate cortices. Again, the sagittal nature of the preparations prevented us to assess the medial extent of their frontal distribution toward the gyrus proreus. Finally, a thorough survey of the entire neocortex disclosed that scattered spindle cells are present in regions where they have not been observed previously in hominids. Thus, we observed isolated examples of typical spindle cells in the inferior temporal cortex, close to its junction of the entorhinal cortex, a few located in the inferior surface of the occipital region in the ecto- and suprasylvian cortex, and in the depth of the paralimbic lobe. Some are also present in the posterior segment of the cingulate and retrosplenial cortex, whereas they are very rare in the temporal extension of the insula and appear to be absent from the rest of the frontal, parietal, and temporal neocortex. The presubiculum also contains a few examples of typical spindle cells, as is the case in humans. Altogether, when considered in the context of our previous data on hominids, the present observation of the Von Economo spindle neurons in a balaenopterid provides an interesting case of convergent evolution of a distinct cell type in the neocortex of highly divergent species.

Overview of layer II clustering. A remarkable feature of the neocortex of *M. novaeangliae* and of *B. physalus*, which in fact can be observed by simple ocular inspection of histologic sections, is the presence of clumping or clustering of neurons in layer II across a very large extent of temporal and occipital cortex. Depending on the plane of section and the region of cortex considered, these clusters can be of variable size and density (Fig. 17A). These clusters are seen in the posterior third of the cortex lining the paralimbic fissure, and along the posterior (occipital) extensions of the ectosylvian, suprasylvian, and lateral gyri (Fig. 2A). They also occur in the anterior insula, as previously described in the bottlenose dolphin (Jacobs et al., 1984; Manger et al., 1998). In some instances, these clusters are larger (>30 cells in some cases) and comparable to the entorhinal cortex layer II islands (Fig. 17B and C; see also Fig. 6I), exhibiting almost cell-free spaces in between them, whereas elsewhere they are made of small aggregations of tightly packed neurons (usually 10–15 cells) linked by bridging regions containing fewer neurons (Fig. 17D). In the latter case, the layer II clusters align with small groups of layer V large pyramidal neurons, seemingly defining a columnar arrangement of the cortical structure (Fig. 17D). Small layer II clusters are present in the posterior two-thirds of the insula in front of the central sulcus, in the cortex of the paralimbic fissure, and toward the lateral aspect of the temporo-occipital cortex. Larger clusters with parallel clumping of layer V neurons are observed in the medial third of the occipital cortex along the inferior extension of the ectolateral and suprasylvian fissures, as well as in the ventral third of the anterior insula and in the frontoinsular cortex (Fig.

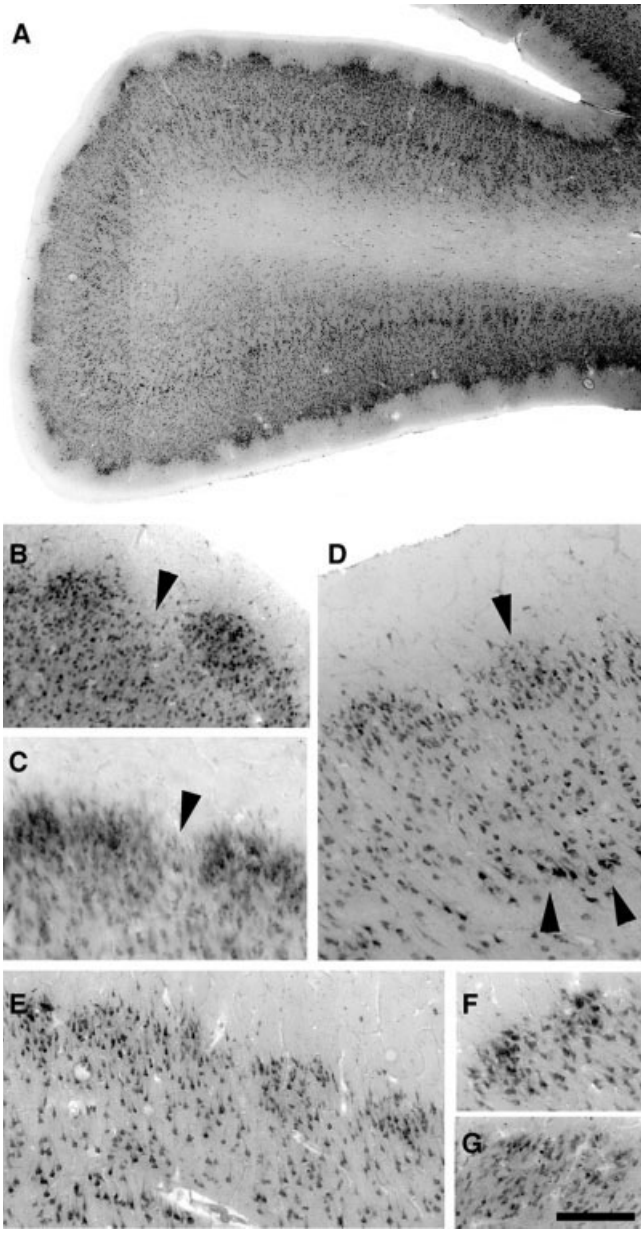

Fig. 17. Neuronal modular arrangement in layer II in the humpback whale cerebral cortex. **A** shows the typical organization of these modules in the occipital cortex. Some of these clusters are large (**B**) and resembles those in the entorhinal cortex (**C**; see also Fig. 6I). Some clusters are more cell-sparse, especially laterally in the occipital cortex (**D**). The anterior segment of the insula contains many clusters as well (**E–G**). These modules are smaller posteriorly (E) and become larger toward the frontoinsular cortex (F and G). Scale bar = 700 μm (A); 150 μm (B–G).

17E–G). The cortex on the lateral aspect of the hemisphere does not show such arrangement in layer II. It was not seen in the lateral and suprasylvian gyri on top of the hemisphere, or in the magnocellular region and the lateral and polar aspects of the frontal lobe. If the later regions represent primarily what is considered to be the motor and sensory domains of the cetacean neocortex, then this particularity of layer II may be present only within certain types of association cortex in mysticetes. Layer II clustering is not present in the neocortex of odontocetes outside of the anterior insula.

Comparison With Other Cetacean Species

Fin whale. Surface samples were taken from one hemisphere of the fin whale to compare targeted cortical regions with the same locations in the humpback whale brain. Our goal was to ascertain that comparable patterns of cytarchitecture were present in the fin whale across the cortex, with a particular emphasis on possible primary motor and sensory regions and cytorachitectural specializations observed in the humpback whale. Thus, blocks were obtained from the occipital and frontal convexity, the lateral gyrus, the ectosylvian cortex, the posterior paralimbic cortex, the anterior insula, the anterior cingulate cortex, and the frontopolar cortex. Although these materials were not of the same quality as the celloidin-embedded sections from the humpback whale, owing probably to the postmortem condition of the specimen, we were nevertheless able to confirm several aspects of the cytoarchitecture of the fin whale cerebral cortex.

The frontopolar and anterior cingulate cortex of the fin whale show relatively sparse layers with an absence of distinct clustering and few large pyramidal cells in layer V. Comparatively to the humpback whale, layer VI appears wider and heterogeneous (Fig. 18A and B). We were able to confirm in the fin whale the presence of many spindle cells in the anterior insula, anterior cingulate cortex, and frontopolar cortex (Fig. 18A and B, and below). The cortex of the medial frontal convexity shows a gigantocellular field similar to that in the humpback whale, also bordered laterally by a region with smaller-size pyramidal cells (Fig. 18C). The paralimbic cortex of the fin whale is comparable to that in the humpback whale with cell-sparse layers II and III, relatively small pyramidal cells in layer III, and intermediate-size pyramidal neurons in layer V, with occasional larger cells and no cell clusters (Fig. 18D). The occipital (posterior ectolateral in this case) cortex of the fin whale is characterized by the same clustering of layer II as in the humpback whale (Fig. 18E). It is thus likely that a comparably large expanse of cortex presents with this particularity in the fin whale. In addition, this dorsomedial occipital region in the fin whale also shows magnocellular pyramidal cells in layer V, as is the case in the humpback whale. The middle tier of the lateral gyrus and the ectosylvian cortex exhibit cellular organization patterns comparable to those observed in the humpback whale for the possible primary visual and auditory field, respectively. The hippocampus of the fin whale is comparably diminutive as in the humpback whale.

As in the humpback whale, the fin whale spindle cells reside in layer V, are very elongated and large, and are found preferentially toward the crown of gyri (Fig. 19A and B). Whereas it was not possible to perform such a detailed survey of their distribution as in the humpback cerebral cortex, it is nevertheless clear that spindle cells show higher densities in the insular cortex than in the other locations, the frontopolar region having the least. Isolated spindle cells also occur in other cortical regions such as the paralimbic, occipital, and ectosylvian cortices, as in the humpback whale. These observations suggest that spindle cells are likely to be a characteristic

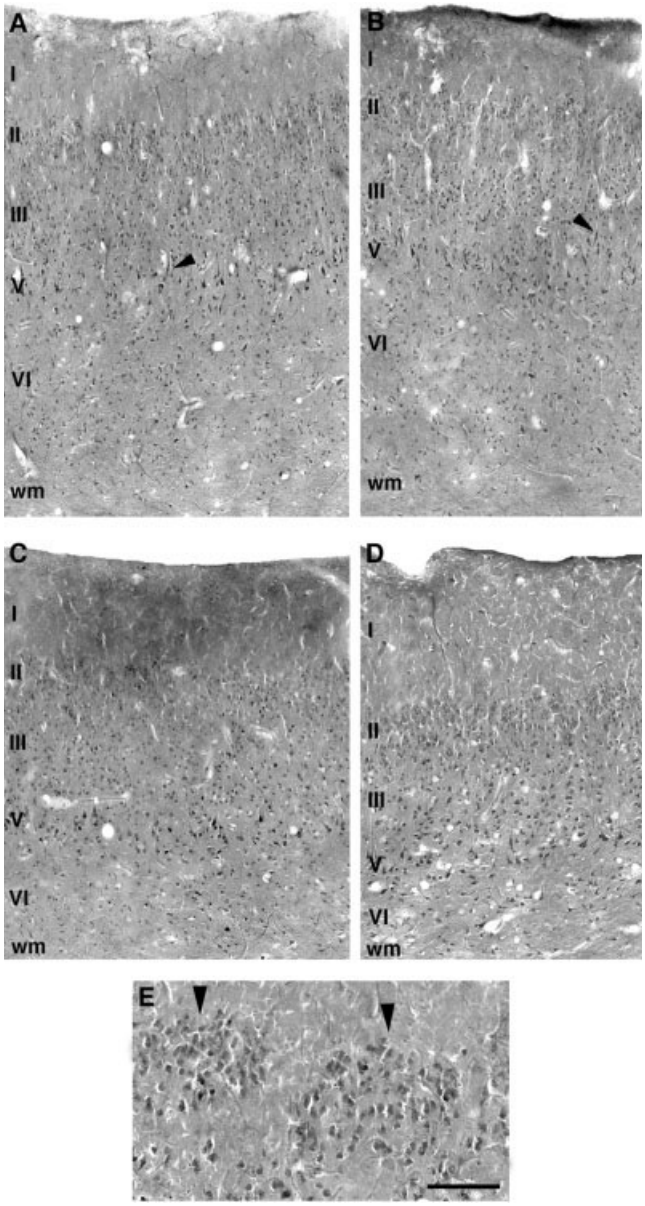

Fig. 18. Cytoarchitecture of the neocortex of the fin whale. The structure of the neocortex in *B. physalus* appears overall similar to that in *M. novaeangliae*. From the available samples, the fin whale neocortex appears slightly thicker than that of the humpback whale and shows lower neuronal densities. **A:** Anterior cingulate cortex. **B:** Anterior insula. Note the occurrence of a few spindle cells in these regions (arrowheads). The lateral frontal convexity (**C**) shows relatively small neurons in layer V as in the humpback whale. The paralimbic cortex displays a clear clustering of large layer V neurons (**D**). The occipital cortex also exhibits a modular organization in layer II (**E**; arrowheads). Layers are indicated by Roman numerals; wm, white matter. Scale bar = 250 μm (A–D); 150 μm (E).

feature of the cerebral cortex of balaenopterids, and possibly of all mysticetes.

Odoncetes. We had the opportunity to study specimens and samples from a number of odontocetes and

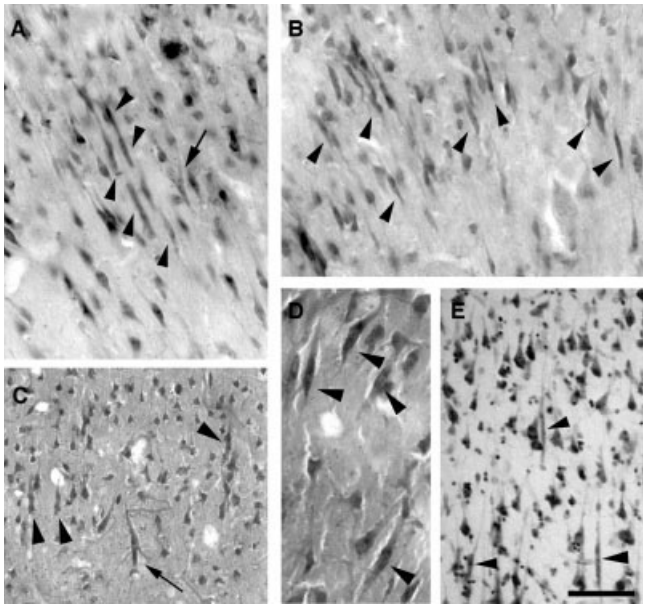

Fig. 19. Spindle cells in the anterior cingulate cortex (**A**) and insula (**B**) of the fin whale, and in the anterior cingulate cortex of the sperm whale (**C**) and insula of the killer whale (**D**). The spindle cells (arrowheads) in these species exhibit a similar morphology as seen in the humpback whale and are present in the same cortical regions (see Figs. 15 and 16). A few had a forked morphology with a dividing apical dendrite (arrows in A and C). The sperm whale and killer whale had slightly lower densities of spindle cells overall (C and D). The spindle cells in cetaceans tended to be less slender than those seen in humans (**E**, for comparison). Scale bar = 100 μm (A and B); 120 μm (C and E); 60 μm (D).

reporting the detailed cytoarchitecture of these species is beyond the scope of this study, as substantial differences in cortical organization is known to occur among cetacean species (Hof et al., 2005). Rather, we focused, as for the fin whale, on target regions in specimens from which series of optimal preparations, comparable in quality to that from the humpback whale specimen, could be obtained (*T. truncatus*, *S. coeruleoalba*, *D. leucas*, and *P. phocoena*). Details from surface samples of matching cortical regions from other odontocete species are discussed as relevant.

Two major differences between odontocetes and mysticetes were observed in the present study. First, none of the available odontocete species show layer II clustering in the occipital cortex. Considering that we had access to species representing all major families of odontocetes, it may be inferred that these occipital layer II clusters is a mysticete specialization. Second, spindle cells were notably absent from the regions where they are abundant in the humpback and fin whales in all odontocetes but two species: the sperm whale and the killer whale. It is interesting to note that these two species, a physterid and a delphinid, respectively, have the largest brains among odontocetes in absolute size, yet by far not the highest encephalization quotients (Marino, 1998; Marino et al., 2004; Hof et al., 2005). In both the sperm whale and the killer whale, spindle cells are quite abundant in layer V of the anterior insula and anterior cingulate cortex (Fig. 19C and D), but much fewer are observed in the frontopo-

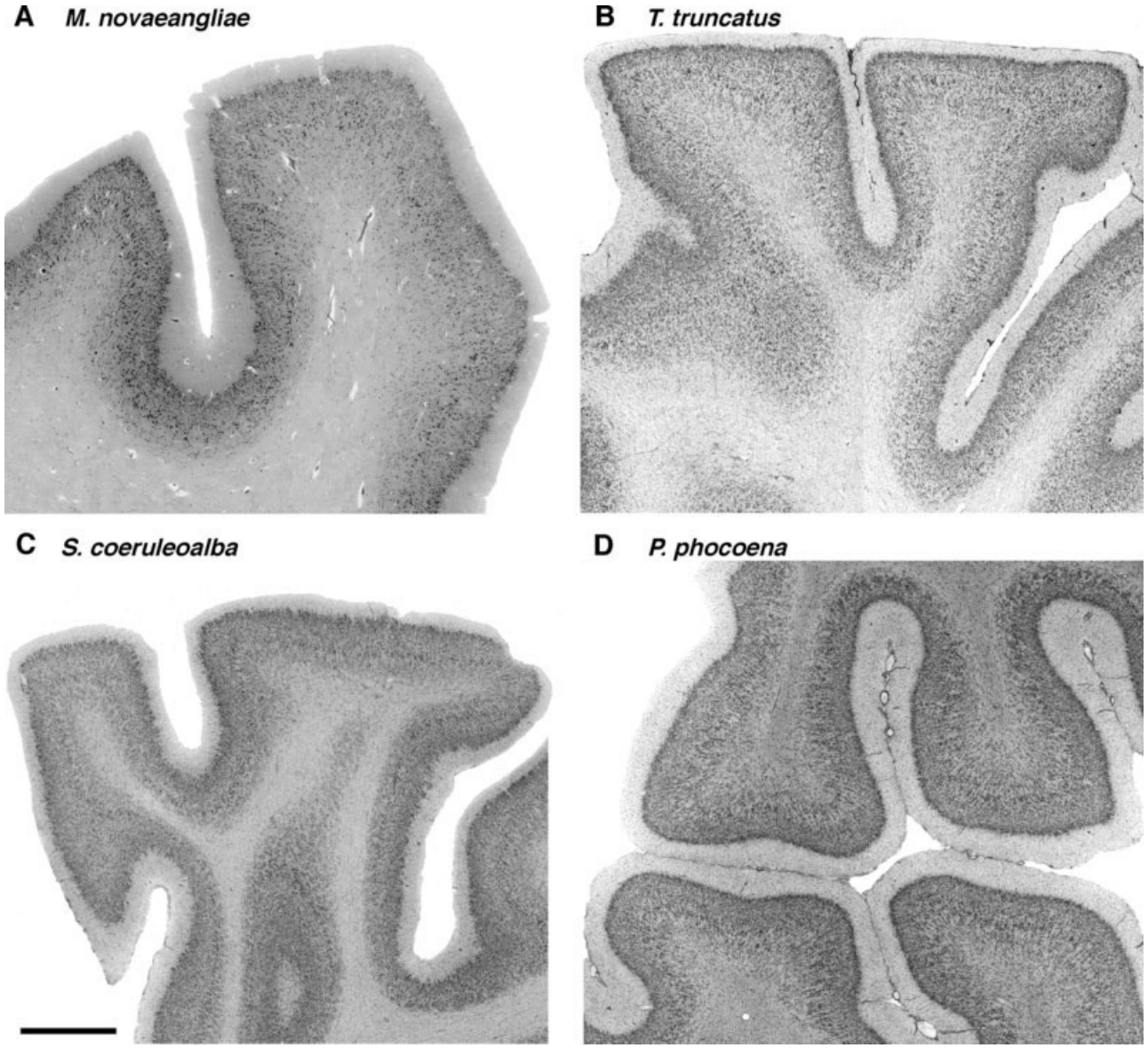

Fig. 20. Comparison of neocortical organization in the humpback whale (**A**) and three smaller odontocetes: the bottlenose dolphin (**B**), the striped dolphin (**C**), and the harbor porpoise (**D**). The anterior cingulate cortex is shown as an example in each species. The midline is up and ventral is right on B and C. The lower bank of the splenial fissure is shown on A, and on D, the lower and upper banks of the intercalate sulcus are visible in the sagittal plane. Note the much lower neuronal density in *Megaptera* compared to all three odontocetes. The striped dolphin and harbor porpoise have the highest neuronal densities. Scale bar = 1.3 mm.

lar cortex. As in the mysticetes, occasional isolated spindle cells are present in other cortical regions. In the two odontocetes, they appear somewhat thicker than in *Megaptera*, with less-well-visible dendrites. It is, however, possible that these morphological differences is due to artifacts of preparation in these less optimal materials. A comparison with spindle cells from the human anterior cingulate cortex is shown in Figure 19E.

Overall, the Bauplan of the cerebral cortex appears to be constant across cetacean species based on the present specimens, in the sense that a comparable distribution of identifiable cortical domains exists in all of the species we investigated, independent of obvious differences in cytoarchitecture among species. The major differences in cortical histologic organization among cetaceans are related to the local cellular densities across cortical field as well as the size of the neurons, particularly visible between *Megaptera* and small-bodied odontocetes (Figs. 20 and 21). As expected, species with larger brains have much lower cellular densities and larger neurons compared to species with small brains (for quantitative data, see Poth et al., 2005), resulting in much variability in cytarchitecture. Such differences can be appreciated when comparing topographically similar cortical locations in species such as *T. truncatus*, *S. coeruleoalba*, or *P. phocoena*, to *M. novaeangliae* and *P. macrocephalus*

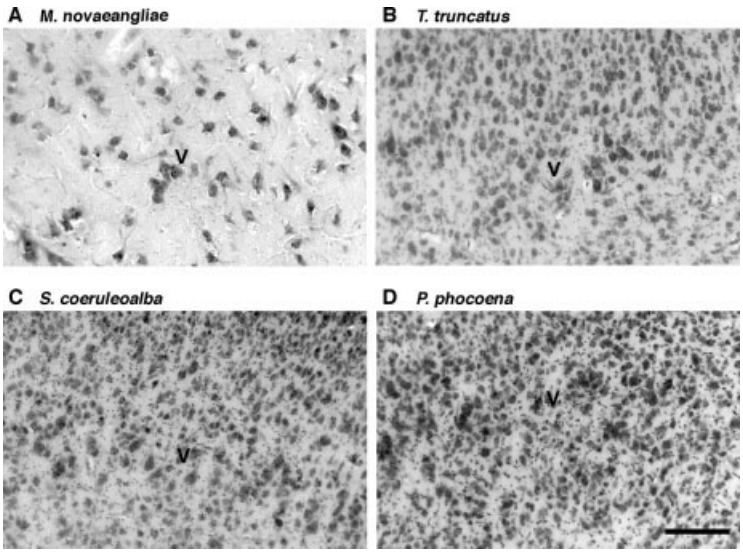

Fig. 21. Neuronal densities at the border of layers III and V in the frontal polar cortex of the humpback whale (**A**), the bottlenose dolphin (**B**), the striped dolphin (**C**), and the harbor porpoise (**D**). Note the low density of neurons in the humpback whale and the tendency of layer V pyramidal cells to form little clusters. Layer V is identified on each panel. Scale bar = 200 μm.

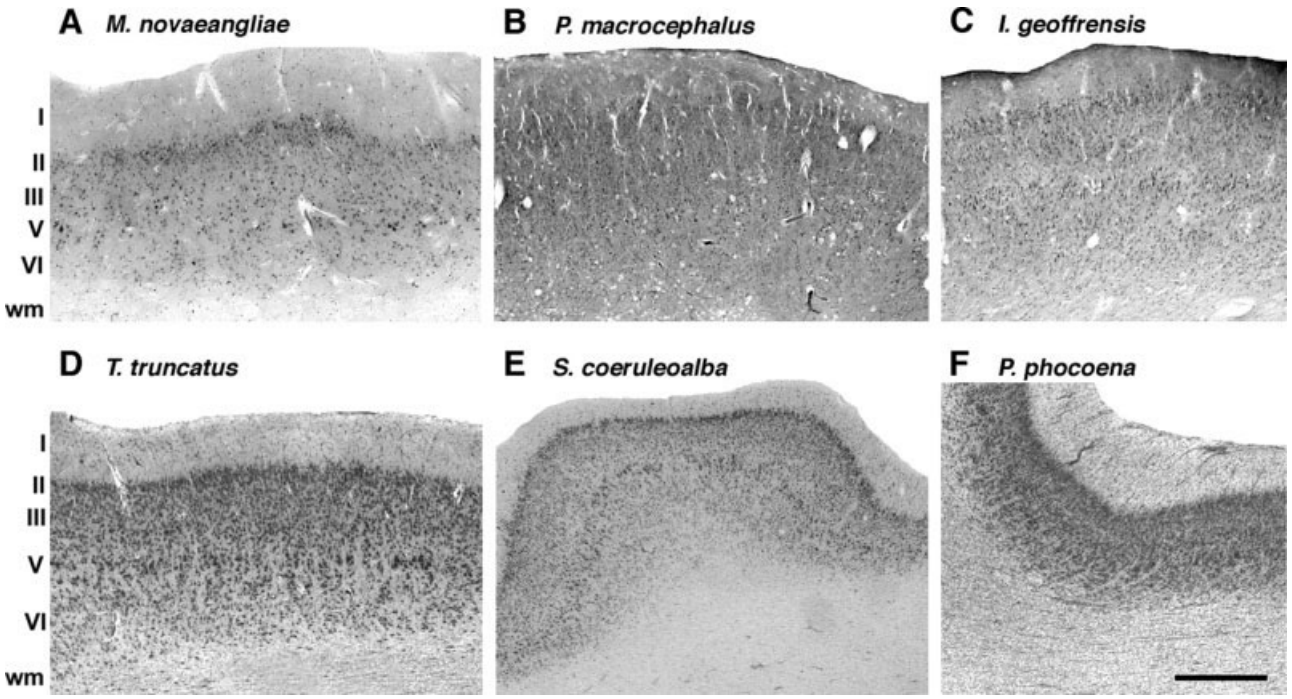

Fig. 22. Comparative structure of the lateral frontal cortex corresponding to the putative somatosensory cortex in the humpback whale (**A**), the sperm whale (**B**), the Amazon river dolphin (**C**), the bottlenose dolphin (**D**), the striped dolphin (**E**), and the harbor porpoise (**F**). In all of these species, this region is characterized by relatively few large neurons in layer V. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 400 μm.

(Figs. 22–25). All species had a recognizable gigantocellular field in the medial frontal convexity, next to a lateral domain exhibiting slightly smaller layer V neurons. Roughly, the situation of this magnocellular "core" corresponds well to the maps proposed by Kojima (1951) for the sperm whale across species as diverse as *M. novaeangliae*, *T. truncatus*, *S. coeruleoalba*, *P. phocoena*, *D. leucas*, *I. geoffrensis*, and *K. simus*. Comparably, a parvocellular domain of cortex occurs lateral to it in all species, with only sparse large layer V pyramidal

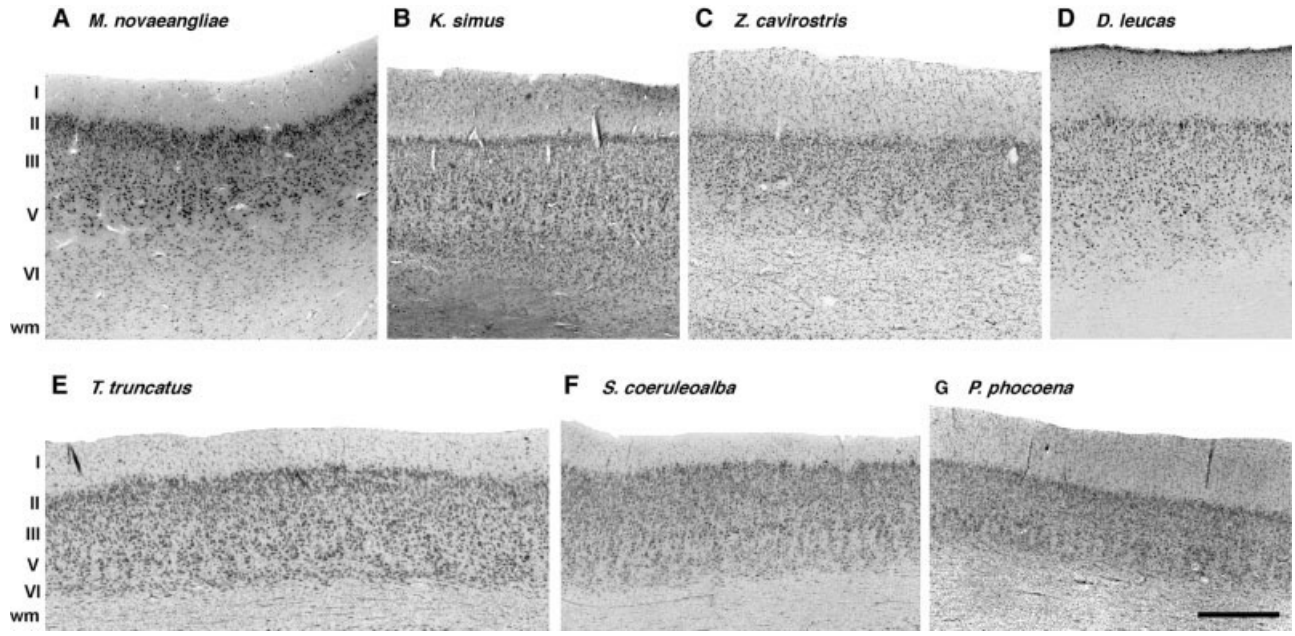

Fig. 23. Comparative structure of the cortex in the middle lateral gyrus at a level that may correspond to the primary visual cortex in the humpback whale (**A**), the dwarf sperm whale (**B**), the Cuvier beaked whale (**C**), the beluga whale (**D**), the bottlenose dolphin (**E**), the striped dolphin (**F**), and the harbor porpoise (**G**). In all of these species, this region is characterized by alternating neuronal modules forming columns and patches of neuropil in layers V and VI. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 400 μm.

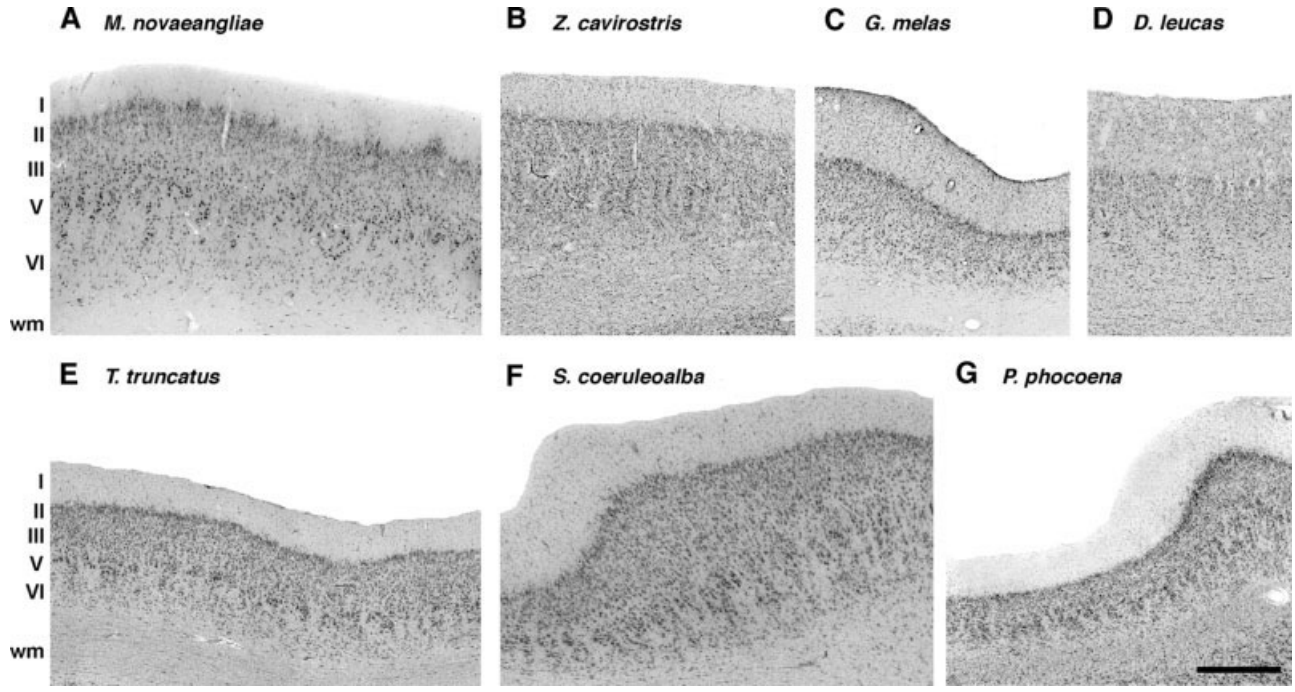

Fig. 24. Comparative structure of the middle suprasylvian cortex possibly showing the primary auditory cortex in the humpback whale (**A**), the Cuvier beaked whale (**B**), the long-finned pilot whale (**C**), the beluga whale (**D**), the bottlenose dolphin (**E**), the striped dolphin (**F**), and the harbor porpoise (**G**). As in the primary visual cortex (Fig. 22), this region is characterized by alternating neuronal modules and patches of neuropil in layers V and VI in all of these species. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 400 μm.

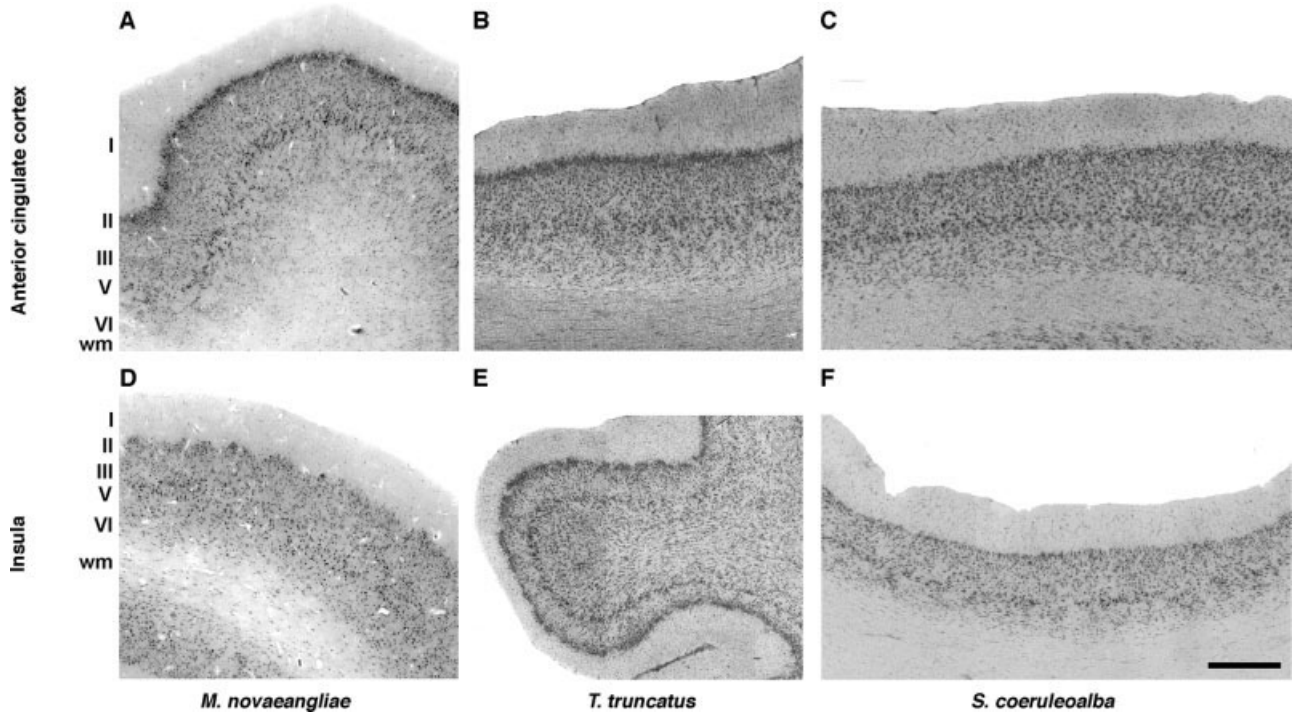

Fig. 25. Comparative structure of the anterior cingulate cortex (**A**–**C**) and insula (**D**–**F**) in the humpback whale (A and C), the bottlenose dolphin (B and E), and the striped dolphin (C and F). The smaller-brained species (F) show a less distinct pattern of layer II modules in the insula, while it is fairly comparable to the bottlenose dolphin otherwise. Layers are indicated by Roman numerals; wm, white matter. Scale bar = 400 μm.

cells (Fig. 22). Similar observations can be made for the visual cortex in the lateral gyrus and the possible supra- and ectosylvian auditory cortices in which a comparable pattern of neuronal stacking and neuropil patches can be observed in layers V and VI (Figs. 23 and 24). Whereas generally the cytoarchitecture of at least the limbic (callosal) aspect of the cingulate and retrosplenial cortex appears to be quite comparable in all of these species, neuronal densities aside, the ventral insula shows less conspicuous layer II islands in smaller-brained species than large-brained ones (Fig. 25). The variability of cytoarchitectural patterns that seems to exist for specific cortical fields, such as the visual cortex (Hof et al., 2005), even among species within a given cetacean family, stands out in comparison with other mammals, such as primates, where a more constant cytoarchitecture is the rule, especially in primary motor and sensory cortices, and certainly deserves further investigation.

DISCUSSION

The present analysis represents one of the rare descriptions of the cortical organization of a mysticete brain. Our findings demonstrate that the cortex of *M. novaeangliae* is characterized by a similar degree of regional complexity as that of smaller-size cetaceans that have been investigated in much greater detail as to the histologic structure of their brain. We observed that the cerebral cortex of *Megaptera* possesses well-defined cortical fields whose boundaries can be established using Nissl-stained preparations with sufficient certainty. The validity of the delineation of such general cortical domains is indeed supported by previous observations mainly in the bottlenose dolphin cerebral cortex, as well as in a few other species (Kojima, 1951; Pilleri et al., 1968; Kraus and Pilleri, 1969; Jacobs et al., 1971, 1979, 1984; Morgane et al., 1980, 1982; Hof et al., 2005). Importantly, while such observations challenge an enduring concept that cetaceans have a comparatively simple and uniform cortical organization (Kesarev, 1971, 1975; Glezer et al., 1988), they also reveal that a generally similar distribution of cortical regions exists in *Megaptera* and odontocetes, at least based on the present comparative survey and our previous study of cortical organization in toothed whales (Hof et al., 2005). The cortical parcellation we propose here obviously outlines only general patterns of cortical organization (Fig. 5). A more detailed and quantitative study of each cortical field would likely show additional subdivisions of the cortex, as could be expected in a mammalian brain, and is beyond the scope of the present report. It would also permit the establishment of topographic boundaries among cortical fields with more precision than was possible in this analysis, as such borders occur as progressive trends rather than sharp changes in cytoarchitecture, especially given that our description is based solely on the Nissl stain, which is known to be less accurate than other methods to reveal the existence and borders of cortical areas. Clearly, our study is limited owing to the rarity of mysticete brain specimens, preventing studying of other individuals from the same species and permitting only some comparison with another balaenopterid. It also presents the inconvenience of being

based only on parasagittal sections, which in some instances made the analysis of some regions impossible.

A large number of studies have used immunohistochemical approaches to map the distribution of cortical fields in many species (e.g., Carmichael and Price, 1994; Hof and Morrison, 1995; Hof et al., 1995, 1996; Van der Gucht et al., 2001, 2006; Kirkcaldie et al., 2002; Baldauf, 2005; Boire et al., 2005; Bourne and Rosa, 2006). While a few reports on restricted domains of the cetacean cortex exist and have explored specific aspects of neuronal typology (Glezer et al., 1992, 1993; Hof et al., 1992, 1999), such methods could not reliably be used in the present study. We could evidently not assess the intraspecific variability in these patterns as they may vary as a function of the individual's age and sex and cannot safely generalize the observed patterns as being representative of all baleen whales, although such generalization appears possible for at least balaenopterids in the light of our observations of samples from a *B. physalus*. In spite of such limitations, we suggest that each "lobar" component is made of many regions that can be grouped in functional assemblages based on the available knowledge of cetacean brain function and organization patterns inferred from other cetacean species as well as from carnivores and primates.

In terms of cortical arealization, the present study was able to determine the possible location on the hemispheric convexity of the principal motor and sensory regions. We confirm the presence of a complex and extensive "limbic" cortex, including the cingulate, retrosplenial, and insular cortices, and show that the frontopolar and orbital cortex is more substantial in cetaceans than generally considered. As in other cetacean species, the hippocampus proper of *Megaptera* is remarkably small, in spite of a comparatively large paleocortex and entorhinal cortex, raising interesting questions about the organization and comparability of the entorhinodentate projections in these species and making the case for possible major differences in cetaceans in cortical processing strategies along circuits known to support fundamental aspects of memory processing in other mammals (Squire and Zola-Morgan, 1991; Zola-Morgan and Squire, 1993). Whereas our description of the limbic and rhinic cortex of *Megaptera* is generally congruent with previous reports (Jacobs et al., 1971, 1979, 1984; Morgane et al., 1982), the distribution of neocortical fields over the hemispheric surface deserves some comparative comments.

The vast expanse of cortex on the parietal and temporal regions of the cetacean brains has been traditionally related to the acoustic capabilities of these species and indeed a number of studies using physiologic mapping have shown the existence of auditory fields and, in odontocetes, echolocation-related fields in the temporoparietal cortex (Zworykin, 1963; Sokolov et al., 1972; Ladygina et al., 1978; Supin et al., 1978; Krasnoshchekova and Figurina, 1980; Voronov et al., 1985). Our results in *Megaptera* show that the cortex of the lateral, supasylvian, and ectosylvian gyri is organized in what seems to represent a system of core and belt regions, with the core displaying a rather thin cortex and the presence of columnar modules in layer VI that are less apparent in the relatively thicker belt areas, comparable to cortical regional hierarchies known to occur in primates and carnivores (Felleman and Van Essen, 1991; Payne, 1993; Scannell et al., 1995). For instance, the middle region of the lateral gyrus shows a cortical type in a position compatible with that of the primary visual cortex in cetaceans (Sokolov et al., 1972; Ladygina et al., 1978; Supin et al., 1978; Garey and Revishchin, 1989; Revishchin and Garey, 1990), surrounded by at least two fields that may be the homologues of areas 18 and 19 sensu lato in carnivores and areas V2 and V3 in primates (Otsuka and Hassler, 1962; Montero, 1981; Sherk, 1986; Payne, 1993; Scannel et al., 1995; Van der Gucht et al., 2001). The lateral suprasylvian and ectosylvian cortex, where auditory fields are known to be located in cetaceans (Sokolov et al., 1972; Ladygina et al., 1978; Supin et al., 1978; Krasnoshchekova and Figurina, 1980; Voronov et al., 1985), is similarly organized in "tiers" in the anteroposterior axis, reminiscent of the organization of the primary auditory and auditory association areas of many terrestrial mammals (Otsuka and Hassler, 1962; Montero, 1981; Sherk, 1986; Payne, 1993; Scannel et al., 1995; Van der Gucht et al., 2001; Lee and Winer, 2005). In the absence of sufficient functional and connectivity data in cetaceans, it is impossible to attribute precise homology to these regions based only on a Nissl stain survey. Such homologies would, however, be supported by the remarkable similarities in the cortical Bauplan that are shared among cetaceans, artiodactyls, and carnivores in terms of the concentric organization of the perisylvian gyri (Morgane et al., 1980). It should be noted, however, that the occurrence in many of the species investigated in the present study of columnar neuron patterns alternating with neuropil patchiness in layers V and VI of these regions argues in favor of such functional attribution as these patterns might be accounted for by specific thalamic visual and auditory afferents.

In a similar fashion, a comparable distribution of the putative motor and somatosensory fields along the cruciate sulcus and what would be homologues of the anterior and posterior sigmoid gyri in cetaceans also supports this view. The localization and extent of the frontal magnocellular and parvocellular core regions reported in this study match physiological data from smaller odontocetes on the primary motor and possible somatosensory regions (Lende and Akdikmen, 1968; Lende and Welker, 1972), as well as the histologic observations of a magnocellular domain in another large-brained species, *P. macrocephalus* (catodon in Kojima, 1951). These generic patterns also suggest that while the cetacean cortical organization can be directly compared to that of many terrestrial species as far as regional distribution is concerned, similar functions across species are subserved by considerably different cellular and laminar features, as well as absolute volume of cortex devoted to a given function. Compared to nearly all terrestrial mammals, most cetaceans have clearly evolved extended parietal and temporal regions, the purpose of which remaining the domain of speculation. Furthermore, our data indicate that a substantial variability in cytoarchitectural patterns exists even for primary cortical fields among cetacean species, and certainly between odontocetes and *Megaptera*, whereas more consistent laminar organization would have been expected based on our knowledge of such regions in terrestrial species. Such differences may underlie unsuspected variability in functional and behavioral aptitudes in obligatory aquatic species that

are in most cases poorly documented and understudied. They also point to the fact that considerably different strategies in terms of cortical typology and wiring can be used to support similar functions across mammals.

In this respect, it is worth noting that the cerebral cortex of *Megaptera* stands out owing to two remarkable histologic features. One is the modular organization of layer II that is observed over a vast extent of occipital cortex and exists in the fin whale as well. Such layer II islands have been observed in the bottlenose dolphin (Jacobs et al., 1984; Manger et al., 1998), but exclusively in the insula. In *Megaptera*, the domain of insular cortex showing such islands is extended compared to Tursiops. In the occipital cortex, these modules occur in different sizes in an apparently region-specific manner. Whereas their role is unknown as the function of the cortical regions that display them cannot be ascertained, it is nonetheless interesting to note that cortical organization across mammalian orders shows the tendency for modular organization and does so across systems (Krubitzer, 1995). Such modules, whose definition has evolved since their first recognition by Mountcastle (1957, 1978, 1997), represent histologically and physiologically distinct cortical domains of variable size, depending on the region and species considered that occur within a larger functional or cytoarchitectural areas. It is indeed tempting to consider the occipital layer II patchiness of the two balaenopterids as a vehicle for specific cortical connectivity of this region, a possibility that would find homologies for example in the barrel field of murid rodents (Manger et al., 1998), and would point to a particular function of this cortex that would be unique to rorquals as the feature is not shared by any of the odontocetes species available to us. In contrast, the insular layer II pattern appears to be shared among all cetaceans. The very question of the evolution of such modular organization remains open. It is possible that modules are shaped by thalamocortical afferents and length of corticocortical projections. It is known that the thalamocortical projections of cetaceans are likely to rely on a very different wiring than in terrestrial species owing to the absence of layer IV and the very thick layer I (Voronov et al., 1985; Revishchin and Garey, 1990). In addition, the development of small modules forming organized projections may favor local networks that are more cost-effective in terms of energy demands in a very large brain, where intrahemispheric networks, rather than callosal, slower connections, are likely to be opted for (Ringo, 1991; Cherniak, 1994; Ringo et al., 1994). Such constraints would support economy of wiring and efficacy of signaling and in the case of rorquals may be crucial to support an undefined, yet highly specialized, cortical function.

The other histologic specialization of *Megaptera* cerebral cortex was the surprising observation of spindle cells in a comparable set of cortical areas as originally reported in hominids (Nimchinsky et al., 1995, 1999; Allman et al., 2005; Watson et al., 2006). The spindle cells, or Von Economo neurons, were originally observed by Betz in the human cingulate cortex and were fully described by Von Economo (1926), in addition to a number of early reports (Nimchinsky et al., 1995). They were recently reported to occur in the anterior cingulate and frontoinsular cortices of humans and all great apes to the exclusion of other mammals (Nimchinsky et al., 1999). Although this study included a survey of a few delphinids, no mysticetes were included. Our data show unambiguous spindle neurons in layer V of these regions in *Megaptera*, with the difference that their distribution appears to include a larger volume of cingulate and insular cortex. In addition, spindle cells in *Megaptera* occur in regions where they had not been seen in hominids such as the frontal polar cortex, although there is likely no functional or topographic homology between the frontopolar region in mysticetes and hominids, as well as in isolation in many areas throughout the cortex. They were also present, in the same cortical locations, in *B. physalus*, suggesting that their occurrence is probable in all mysticetes.

The function of spindle neurons is not understood. There exists evidence from the human brain that they represent a class of projection neurons that send an axon subcortically and possibly callosally (Nimchinsky et al., 1999; Allman et al., 2005; Watson et al., 2006). Current hypotheses based on available data in human state that spindle cells may provide an output from the anterior cingulate cortex and frontoinsular cortex to prefrontal and temporal association cortices involved in theory of mind. These cells have been found to be highly vulnerable in Alzheimer's disease and other debilitating brain disorders such as autism and schizophrenia (Nimchinsky et al., 1995; Allman et al., 2005), supporting a role in the failure of high-level function such as social conduct, intuition, and judgment in such diseases. Alternatively, or in addition, these layer V neurons are also in a position to project to a variety of subcortical centers such as the amygdala, hypothalamus, and periaqueductal gray, to which the concerned regions of cortex are known to project in primates (Nimchinsky et al., 1999). As such, these neurons may be involved in the control of complex integrations involving emotions, vocalization control, facial expression, or autonomic function as well as regulation of visceral, olfactory, and gustatory functions, as well as visceral functions and alimentary behaviors.

Remarkably, spindle neurons were also observed in the same location in the odontocete species with the largest brains, *P. macrocephalus* and *O. orca*. This suggests that as shown previously in hominids (Nimchinsky et al., 1999), the presence of these cells is not necessarily related to high encephalization quotient, but rather to absolute brain size. Spindle cells are prevalent in species with the largest brains in primates and in cetaceans, but not in the cetaceans with the highest encephalization levels, as they were never seen in smaller-bodied, yet relatively larger-brained delphinids such as the bottlenose dolphin, the Pacific white-sided dolphin, or the tucuxi. Why and how absolute brain size would be the driving force of spindle cell evolution remains to be determined. Indeed, the study of the brain of other taxa not directly related to cetaceans and primates but characterized by large brains, such as the elephant, will be crucial in this context. Considering the presence of spindle neurons in regions of the insula and anterior cingulate cortex from which vocalizations can be elicited in anthropoid primates (Jürgens, 1982), their possible involvement in the control of vocal behavior may be particularly interesting in the context of the species-specific rich repertory of vocalizations and song in cetacean species (Richard et al., 1996; Whitehead, 1998; Mann et al.,

2000; Rendell and Whitehead, 2003; Weinrich et al., 2006), although the regulation of such behaviors in these species likely relies on other systems than in primates. It is also possible that their presence correlates with certain aspects of social patterns such as gregariousness, which is known to increase brain size in ungulates, the close relatives of cetaceans (Pérez-Barbería and Gordon, 2005). Gregariousness can be seen as a measure of sociality and this would in turn be consistent with a proposed role of spindle cells in social cognition (Allman et al., 2005). Finally, many aspects of cortical and subcortical connectivity are likely to differ in cetaceans from terrestrial species as exemplified by unique patterns and hemispheric regulation of sleep and wakefulness (Manger et al., 2003). Whether spindle cells contribute to such functions and behavioral patterns, while representing interesting hypotheses, evidently remains fully speculative. Their occurrence should, however, not be deemed a by-product of developmental consequences of increasing brain size (Finlay and Darlington, 1995; Finlay et al., 2001). Whereas developmental constraints clearly influence the evolution of the brain or of its cellular components, it cannot be simply said that changes in structure conditioned by development necessarily precede functional use because ontogenetic and adaptationist scenarios are likely to act in synergy rather than as alternatives. This is exemplified by the fact that in both cetaceans and hominids, spindle cells are a feature of the largest brains within the orders but not across, as many delphinids do not have spindle cells, yet their brains are substantially larger than the human brain.

From an evolutionary point of view, it is interesting to consider that in the primate lineage, spindle neurons probably first appeared in the common ancestor of hominids about 15 million years ago, as they are observed only in extant great apes, including humans, but not in lesser apes and other primates. In cetaceans, they evolved earlier than in primates, as they were likely present in the early Miocene, about 22 million years ago, if they were present in the ancestral balaenids, and possibly even before that, in the common ancestor of mysticetes (Fordyce and Barnes, 1994; Geisler and Sanders, 2003). In the case of *Physeter*, spindle cells have the potential to have emerged in the middle of the Oligocene about 30 million years ago, physeterids being an ancient family of toothed whales (Fordyce and Barnes, 1994). Considering recent evidence that stem mysticetes were toothed predators (Fitzgerald, 2006), it may be that spindle cells were present in the cerebral cortex of the ancestral forms of all modern odontocetes and mysticetes and were retained only in those with the largest brains during their evolution. It is also possible that in cetaceans, the spindle cells have evolved several times independently in different lineages within each of the two suborders, first in physeterids, then possibly in balaenids (although the present study lacks a balaenid representative to ascertain their presence in this family), and finally at about the same time in balaenopterids and in large delphinids such as Orca (Fordyce and Barnes, 1994). At the time of the third possible emergence of spindle cells in cetaceans, they were appearing in the ancestor of great apes, presenting an interesting and rare case of parallel evolution characterized by the occurrence of a unique morphologic type of projection neurons, remarkable for its discrete distribution in a few, functionally related, cortical regions in a very restricted yet significant number of highly social species, all characterized by a relatively recent evolution, a slow maturation, a low reproduction rate and few offsprings (i.e., K-selective species), a very large brain and a large body size within their groups.

Whereas the role(s) played by these neurons in the cetacean species in which they occur cannot be decided, their presence in some of these species and their distribution in specific cortical areas directly comparable to the situation in hominids are consistent with the substantial evidence for behavioral and social complexity in cetaceans (Marino, 2002b). Much data on learning, memory, and artificial language comprehension point to the fact that species such as the bottlenose dolphin are quite comparable in these domains of cognition to chimpanzees (Herman, 2002). Bottlenose dolphins also demonstrate remarkable abilities related to self-awareness, such as mirror self-recognition, a rare case among mammals that was known only from hominids until recently, as well as self-monitoring (Reiss and Marino, 2001; Smith et al., 2003). Indeed, most of our knowledge on cetacean cognition and behavior is inferred from investigations of delphinids, in particular *Tursiops*, which lack spindle cells. As such, the present observations suggest that large-brained species may be characterized by a repertoire of cognitive capacities that differ from and even extend beyond those of smaller delphinids. In spite of the relative scarcity of information on many cetacean species, it is important to note in this context that sperm whales, killer whales, and certainly humpback whales exhibit complex social patterns that include intricate communication skills, coalition formation, cooperation, cultural transmission, and tool usage (Connor et al., 1992; Clapham and Mead, 1999; Clapham, 2000; Rendell and Whitehead, 2001, 2003; Valsecchi et al., 2002; Krutzen et al., 2005). It is thus likely that some of these abilities are related to comparable histologic complexity in brain organization in cetaceans and in hominids.

In conclusion, our observations on the cerebral cortex of *M. novaeangliae*, as well as *B. physalus*, demonstrate the existence of consistent patterns of organization with a number of odontocetes. However, both balaenopterids show several departures from the odontocete cytoarchitecture, in particular the presence of modular arrangements of specific groups of neurons over vast domains of cortex that in toothed whales are much more restricted, and the occurrence of large numbers of spindle cells, the latter making a case of parallel evolution with hominids. Cetacean and primate brains may be considered as evolutionary alternatives in neurobiological complexity and, as such, it would be compelling to investigate how many convergent cognitive and behavioral features result from largely dissimilar neocortical organization between the two orders. Our data also show that the cytoarchitecture of the cetacean cerebral cortex is likely to be more variable across species than expected. In view of the fact that many cetacean species are naturally elusive, poorly documented, and often endangered, the present findings also provide an anatomical framework for further correlative and comparative investigations of brain and behavior in cetaceans.

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